THE UNIVERSITY OF MANITOBA

A CONFORMATIONAL STUDY OF 2'-O METHYL URIDINE, 2'-O METHYL CYTIDINE AND 2'-O METHYL ADENOSINE BY PROTON MAGNETIC RESONANCE SPECTROSCOPY

BY

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ABSTRACT

A proton magnetic resonance study of 2'-O methyl uridine (Um) and 2'-O methyl cytidine (Cm) {and a first order study of 2'-O methyl adenosine (Am)} has been carried out in aqueous solution, and at both ambient and high temperature.

Um and Cm are shown to exist in the anti conformation. They both exist in a $C_{(2')}$ endo $\rightleftharpoons$ $C_{(3')}$ endo equilibrium with a slight shift towards the $C_{(3')}$ endo mode. The gauche-gauche rotamer is preferred by the exocyclic CH_2OH group.

2'-O methylation is shown to have little effect on the furanose conformation and the population of exocyclic rotamers about the $C_{(4')}$ - $C_{(5')}$ bond of adenosine.

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CHAPTER I

Introduction

INTRODUCTION

The involvement of nucleic acids in genetics has been well acknowledged and has stimulated an active research in nucleic acids in recent years.

Nucleic acid can be visualized as a single stranded copolymer of nucleotides that contain mainly five heterocyclic bases (adenine, guanine, cytosine, uracil and thymine), a D-ribose or D-deoxyribose attached to the N(9) of the purines and N(1) of the pyrimidines, and a phosphate group attached to the 3' position or the 5' position of the pentose. The structures of the five basic nucleosides are shown in Figure 1.

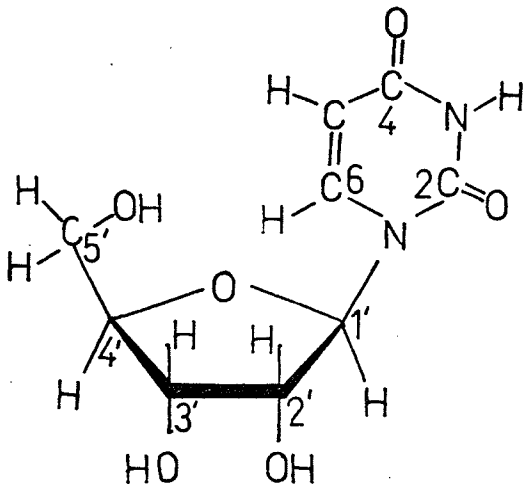
DNA (deoxyribonucleic acid) contains four major nucleosides: deoxyadenosine, deoxyguanosine, deoxycytidine and deoxythymidine; while RNA contains the four major nucleosides: adenosine, guanosine, cytidine and uridine.

The basic polymeric structure can be modified by the addition of substituents at specific locations. Such modifications give rise to a class of nucleosides known as minor, or modified nucleosides.[1]

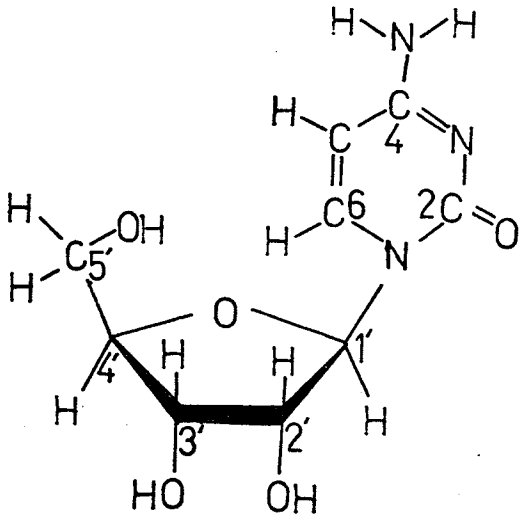
The first modified nucleoside, 5-methylcytidine, was detected by Hotchkiss in 1948.[2] Since then, five modified nucleosides in RNA have been identified. Most of the recent research on nucleic acid has been centered on t-RNA. The complete sequence of fifteen t-RNA species are known. Many modified nucleosides have been detected in t-RNA;

Figure 1a

Structural formula of Uridine (U) and Cytidine (C).


O
H
C
4
N
H
H
C
6
2C
O
H
N
H
OH
5'
H
O
1'
4'
H
H
H
H
3'
2'
H
HO
OH

U

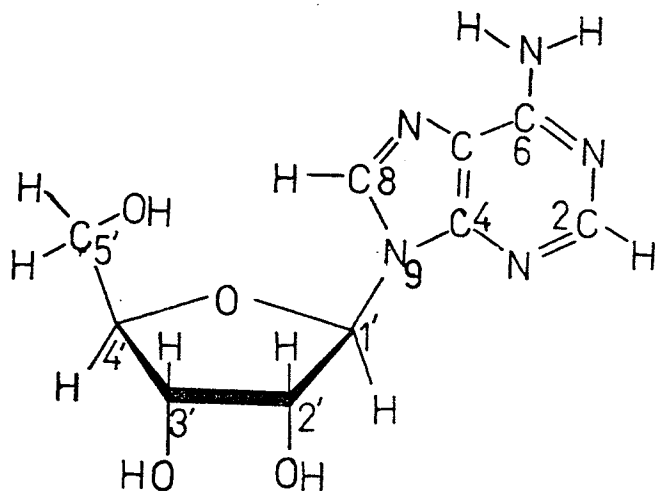

H
N
H
H
C
4
N
H
C
6
2C
O
H
N
H
OH
5'
H
O
1'
4'
H
H
H
H
3'
2'
HO
OH

C

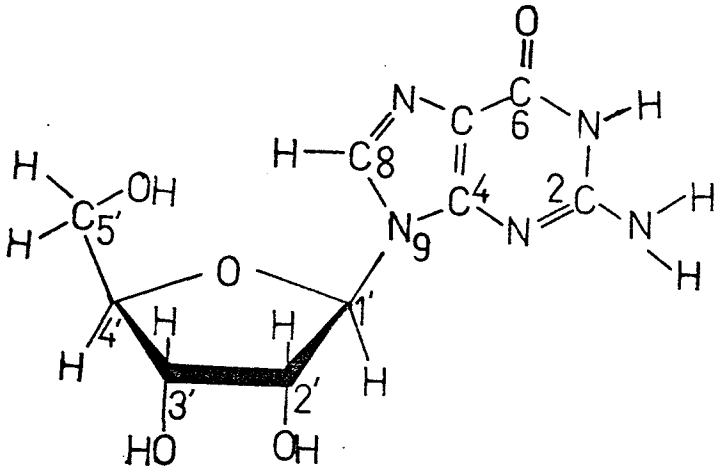
Figure Ib

Structural formula of Adenosine (A),

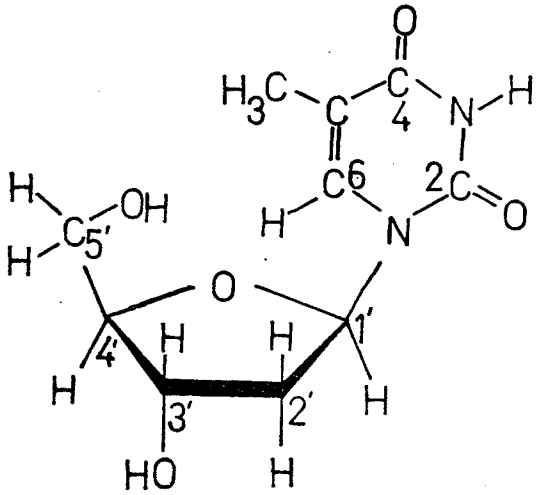
Guanosine (G) and Thymidine (T).



A



G



T

most of these minor nucleosides are in the methylated form, either in the bases or in the 2'-O region of the sugar ring.[3] The structures of Um, Cm and Am are shown in Figure 2.

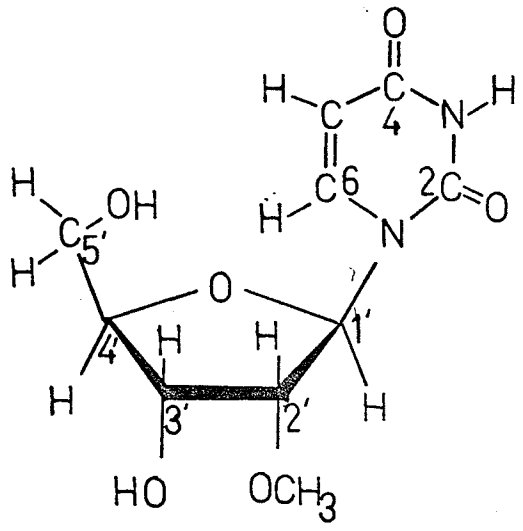
2'-O-Me Adenosine was first isolated by Smith and Dunn[4] in 1959 from RNA of wheat germ and rat liver. Hall[5] also isolated 2'-O-Me A, 2'-O-Me G, 2'-O-Me U and 2'-O-Me C later in 1963. These 2'-O-methylated nucleosides were also found in bacterial and mammalian tissues; the total amount of 2'-O-methylated nucleosides in most analysed samples is of the order of 1% of the total nucleosides.[6]

The 2'-O-methylation of the four principal ribonucleosides is non-random. Each of the 2'-O-methylnucleosides is non-randomly distributed with respect to the normal ribonucleosides in s-RNA and r-RNA.[7,8,9]

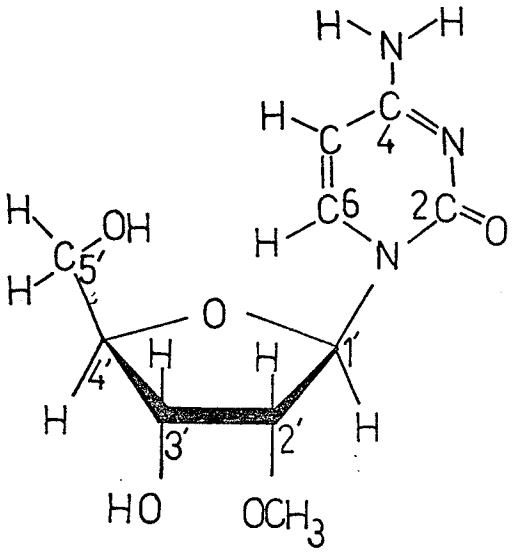
Methylation of t-RNA is believed to occur at the polymer level.[10] Methylases have been isolated from many organisms and their specificity found to be very high.[4] This specificity suggests that methylation must play an important role in the biological activity of the t-RNA, probably in genetics and stabilization of the three-dimensional conformation of the t-RNA molecule as a whole.[11,12,13] But, how the methyl group is affecting the activity of the t-RNA at the molecular level is still obscure. It was suggested that the O-Me group located at the 2' position of a ribonucleoside prevents the -OH group from participating

Figure 2

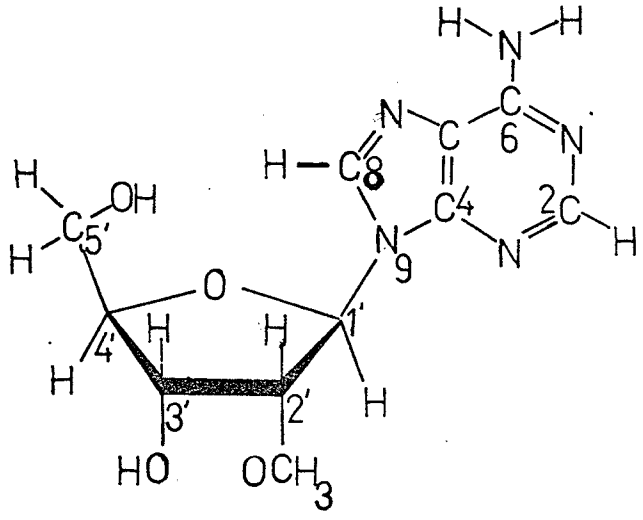
Structural formula of 2'-O Methyl Uridine (Um), 2'-O Methyl Cytidine (Cm) and 2'-O Methyl Adenosine (Am).



Um



Cm



Am

in chemical reactions,[14] for example, formation of cyclic 2'-3' phosphate intermediate during enzyme hydrolysis, thus enzymes like pancreatic RNase cannot hydrolyse bonds formed between 2'-O-Me-U-3' phosphate or 2'-O-Me-C-3' phosphate[6] and the 5'-hydroxyl of an adjacent nucleotide. For the same reason, existence of all the sixteen possible alkali-stable dinucleosides in alkali hydrolysates of ribonucleates from wheat germ[8] and chlorella[9] have been documented. The stability of these dinucleosides is attributed to the occurrence of the 2'-O Me ribose residues in the ribonucleate chain. The 2'-O Me substituent has been found to strongly inhibit the rate at which snake venom phosphodiesterase hydrolyses the internucleoside-phosphodiester bond in alkali-stable dinucleoside phosphates.[7]

Methyl-deficient t-RNAs have been studied by different workers[15,16] and found to have significant differences in coding properties from normal t-RNAs.

Under methylated t-RNA^{phe} has been examined to determine its amino acid-accepting capacity and found considerably less reactive than the normal t-RNA^{phe} in E.coli.[17,18]

So far, no firm conclusion concerning the three-dimensional structure of t-RNA is known. Some workers have studied the structure of poly-2'-OMe adenylic acid at acidic and neutral pH.[19,20] Doubt has been raised

concerning the hydrogen bond donor capacity of the 2'-OH group as an essential requirement for the increased stability of oligo- and polynucleotides relative to the deoxy-compounds. Comparative experiments with 2'-O-methyl oligo- and poly-adenylic acid and the respective unmethylated compounds have been carried out.[14]

However, I feel that in order to understand the role of methylated nucleosides in t-RNA we have to start at the monomer level, studying the three-dimensional structure of 2'-O-methylated nucleosides as compared to unmethylated nucleosides. Then, hopefully, these would lead us to more information about the structure of the dimer, trimer and the polymer.

In this thesis, the three-dimensional structure of 2'-O-methyl uridine (Um) and 2'-O-methyl cytidine (Cm), (partial analysis of 2'-O-me Adenosine (Am) is included), are investigated using high resolution PMR.*

The three-dimensional structure of these compounds can also be studied by other techniques like circular dicroism (C.D.), optical rotatory dispersion (O.R.D.), X-ray diffraction, and ultra-violet spectroscopy (U.V.). A large number of works in this field at the monomer and dimer levels have been done in recent years. However, many limitations in these techniques cause a number of drawbacks in obtaining information about the three-dimensional structure. For example, X-ray diffraction can only be applied to investigate the structure of ribonucleosides

* Proton magnetic resonance

in crystalline form which may deviate from the compound existing in biological systems. O.R.D. and C.D. cannot give information about ring puckering of the sugar.

In recent years, PMR has provided a very useful tool for studying structures and conformations of macromolecules, molecular interactions and molecular processes.[21] It has proved to have a number of advantages over O.R.D., C.D. and X-ray in conformation determination.

The study of the three-dimensional structure of Um and Cm was based on the (a) sugar-base-torsion-angle (SBTA) or glycosidic angle between the sugar and base; (b) the puckering of the furanose ring; and (c) the exocyclic conformation. These features are compared between 2'-O-methylated and non-methylated ribonucleosides.

CHAPTER II

CONFORMATIONAL STUDY OF NUCLEOSIDES

Conformational Study of Nucleosides

A. Planarity of the Base

The planarity of the base moiety of common nucleosides and nucleotides has been studied extensively by X-ray diffraction. It is now known that the ring atoms of the bases themselves are mostly coplanar with displacement less than 0.1 Å.[22] However, the C(1') atom is usually found to be displaced from the base plane by 0.1 to 0.2 Å.

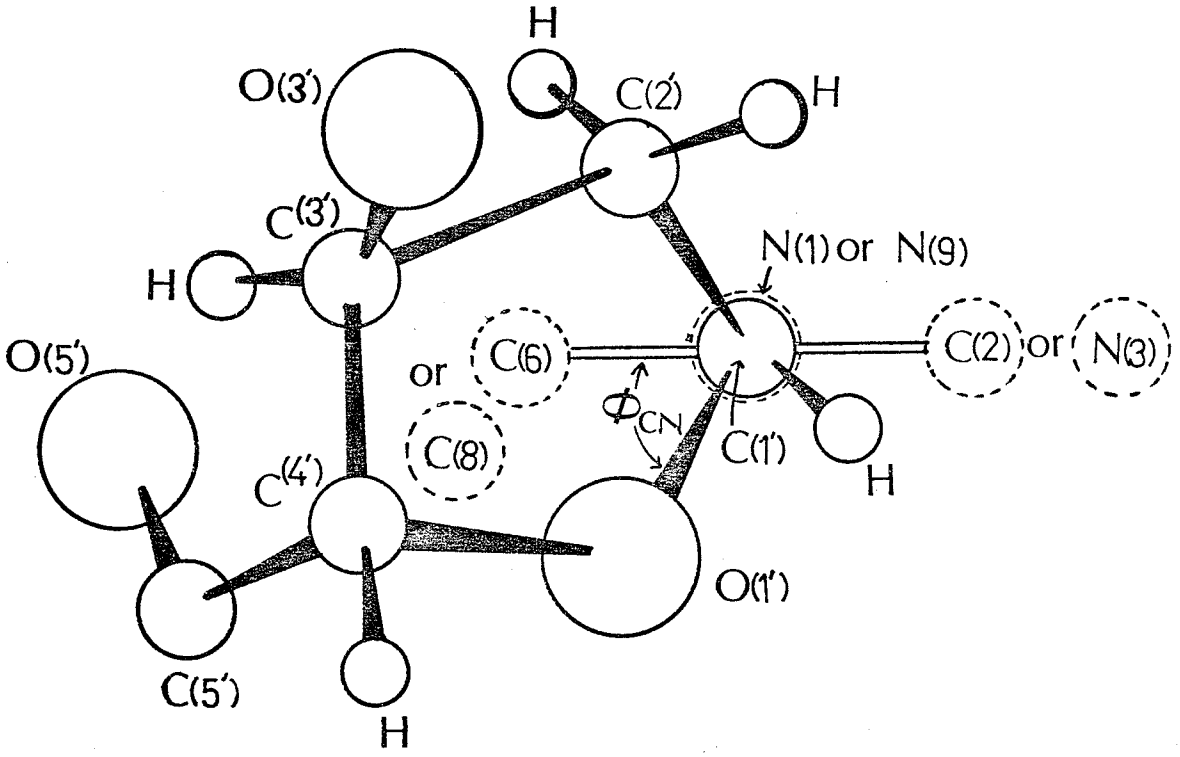
B. Sugar-base Torsion Angle

There is a hindered rotation between the base and the furanose ring about the CN glycosidic bond, that is, C(1') - N(9) bond of purine nucleosides or C(1') - N(1) bond of pyrimidine nucleosides. Donohue and Trueblood first defined the sugar-base torsion angle to describe the orientation of the base moiety with respect to the pentose ring.[23] The illustration of the sugar-base torsion angle is shown in Figure 3, following the definition of Donohue and Trueblood. The two conformations ascribed to nucleosides and nucleotides are defined by two ranges of the torsion angle, ϕ_{CN}, - 30° for the anti conformation and + 150° for the syn conformation. They both have a range of ±45°. It has been found that most of the pyrimidine nucleosides are in the anti conformation in aqueous solution. It is suggested recently that the prime factor that determines the anti and syn conformation in pyrimidine nucleosides is the non-bonding interaction between the base substituent at the ortho-position and the pentose ring. Thus, substituting a bulky group in the C(6) position, for example, 6-methyl uridine[24] can lead to a strong Van der Waals contact between the 6-methyl and the 5'-CH_2OH group or ring protons if the molecule remains in the anti conformation. This strain can only be relieved by assuming a syn conformation instead.

The three most common physico-chemical methods used to obtain information about the torsion-angle of nucleosides

Figure 3

Illustration of the torsion angle in a pyrimidine or purine nucleoside (after Haschemeyer and Rich[76]). The torsion angle ϕCN is the dihedral angle between the plane of the base and the plane formed by the $C_{(1')} - O_{(1')}$ bond of the furanose ring and the $C_{(1')} - N_{(1')}$ bond. The angle is zero when $O_{(1')}$ lies directly in front of $C_{(6')}$ [or $C_{(8)}$ for a purine], and positive angles are measured when $C_{(1')} - O_{(1')}$ is rotated in a clockwise direction when viewing from $C_{(1')}$ to N.


H
O(3')
C(2')
H
C(3')
N(1) or N(9)
H
O(5')
or
C(6)
C(2) or N(3)
C(8)
Φ_{CN}
C(1')
H
C(4')
O(1')
C(5')
H

are X-ray diffraction studies on crystals, ORD-CD studies and NMR studies. Only the NMR studies are discussed in this thesis.

Different approaches have been used to obtain information about anti and syn conformation in nucleosides (nucleotides) based on the NMR technique.

Schweizer, et al[24] have made use of the 2-keto anisotropic effect upon the chemical shifts of the ribose protons to determine the anti and syn pyrimidine nucleoside conformation. The ribose protons of interest are H(1'), H(2'), H(3') and H(4'). They observe a deshielding at H(2') (0.5 - 0.6 ppm) and at H(3') (0.15 - 0.2 ppm) in 6-methyl derivatives of several pyrimidine nucleosides which have syn conformation instead of the normal anti conformation. On the other hand, a shielding effect on H(1') (0.16 - 0.25 ppm) and H(4') (0.17 ppm) is observed. These experiments offer a qualitative determination of syn vs anti conformation.

Dugas, et al[25] observe a similar deshielding effect upon the H(2') and H(3') and a shielding effect upon H(4') in β-cyanuric acid riboside which has an α-keto group in the base lying over the ribose ring, that is, in the syn conformation.

Prestegard and Chan[26] rationalize the slight differences in chemical shifts of the H(6) protons in uridine, dU and 3'Ump on the basis that the chemical shift of H(6) would be sensitive to the variation in the torsion angles due to possible electric field effect and/or magnetic anisotropy

effect of the ether oxygen. Thus, by observing the variation in the chemical shift of the H(6) proton in various pyrimidine nucleoside derivatives can provide a qualitative method of determining the limits of the torsion angles.

Hruska[27] has suggested a way to determine anti and syn conformation in pyrimidine nucleosides by observing the long range coupling between the H(5) and H(1') protons ($^5J_{1'5}$). For coupling over five bonds, the most favorable orientation of the path connecting $H_{(5)}$ and $H_{(1')}$ is the planar 'zig-zag' path, which only occurs in the anti conformation but not in the syn conformation. Thus, the existence of such $^5J_{1'5}$ coupling can be treated as an evidence of the anti conformation.

C. Puckering of the Furanose Ring

In the early sixties, C.D. Jardetzky[28,29] was the first to study successfully the conformation of the furanose ring of nucleosides by applying Karplus relation especially to the $J_{1'2'}$. Since then, the Karplus relation has been used extensively to determine the furanose conformation.[30,31,32,35]

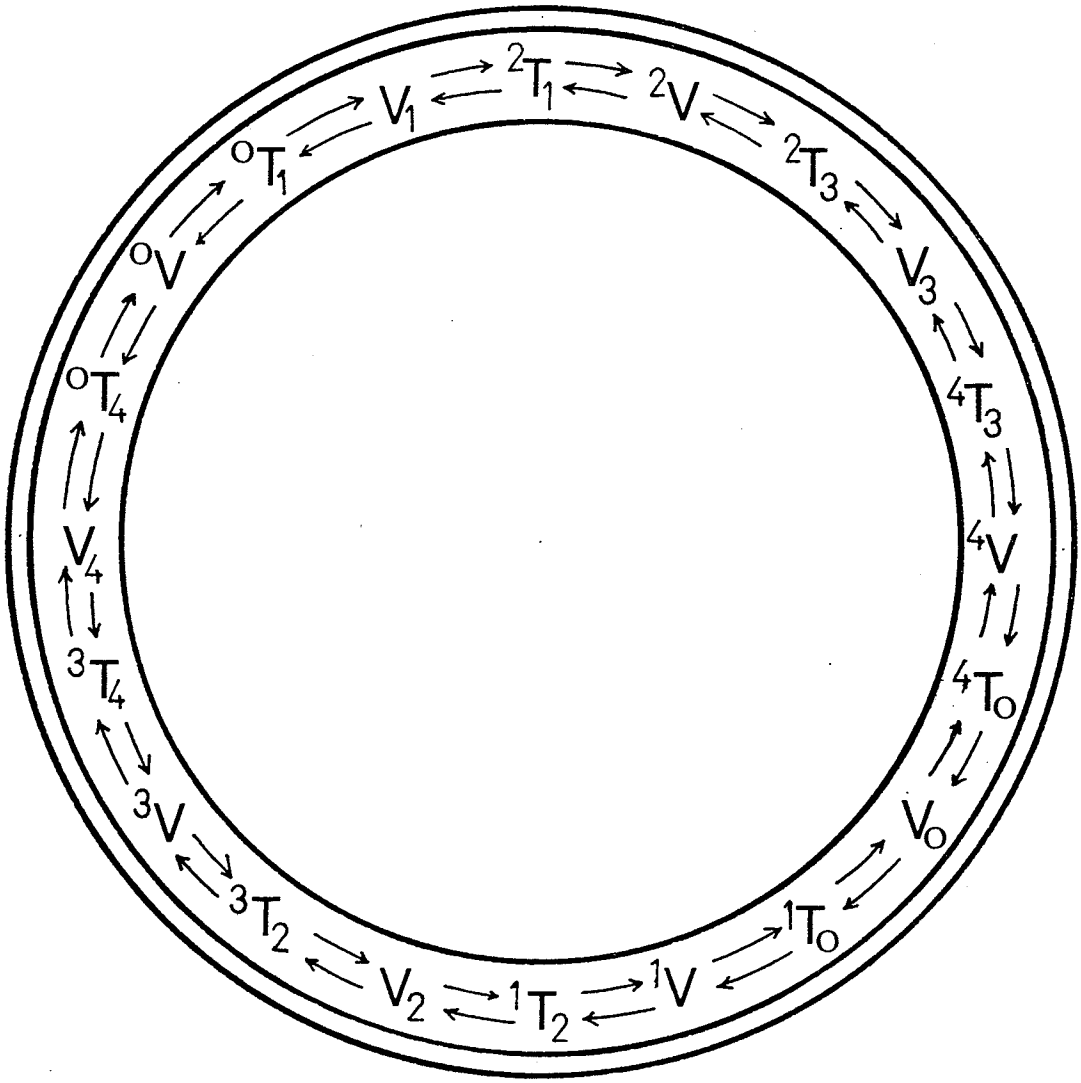
C.D. Jardetzky and M. Smith[32] propose twenty possible conformations of D-ribose and D-deoxyribose in nucleosides and nucleotides. Similarly, Hall[33] et al propose twenty possible conformations based on the conformational features of the cyclopentane ring. They confine discussion only to those furanose conformations having a symmetry relationship with the 'envelope' (V) and 'twist' (T) conformations. The 'envolope' conformer ${}^{n}V$ or V_m has the atom n or m in the endo or exo position respectively with reference to the remaining four ring atoms. The 'twist' conformer ${}^{n}T_m$ has the atom n endo and m exo with reference to the other three atoms. Endo is defined as being on the same side of the $C_{(4')} - C_{(5')}$ bond and exo is on the opposite side. These conformers are represented by the CYCLE of Pseudorotation (Cyclops) in Figure 4. Among all these conformers, the most unstable appear to be the ones in which the $H_{(2')}$ and $H_{(3')}$ are eclipsed.[34]

However, only the interconversion between $C_{(2')}$-endo and $C_{(3')}$-endo is discussed here due to the ambiguity involved in the treatment of the ring puckering as pointed out by Hruska et al.[34]

Vicinal coupling (${}^{3}J_{AB}$) of the fragment H_A-C-C-H_B can be related to the dihedral angle ϕ by the Karplus_

Figure 4

Cycle of Pseudorotation (CYCLOPS) for furanose sugars (after Hall *et al*[33]). The T and V designate the Twist and enVelope conformation respectively.


$^{2}T_{1}$
^{2}V
$^{2}T_{3}$
V_{3}
$^{4}T_{3}$
^{4}V
$^{4}T_{o}$
V_{o}
$^{1}T_{o}$
^{1}V
$^{1}T_{2}$
V_{2}
$^{3}T_{2}$
^{3}V
$^{3}T_{4}$
V_{4}
$^{o}T_{4}$
^{o}V
$^{o}T_{1}$
V_{1}

relation:[36,37]

(1) $J = J^{\circ} \cos^2\phi - C$ for $0^{\circ} \leq \phi \leq 90$

(2) $J = J^{180} \cos^2\phi - C$ for $90^{\circ} \leq \phi \leq 180^{\circ}$

For an unsubstituted fragment, $J^{\circ} = 8.5$ Hz, $J^{180} = 9.5$ Hz and $C = 0.3$ Hz. The choice of the appropriate values of J° and J^{180} for different substituents in the H_A-C-C-H_B fragment limits the validity of the Karplus relation significantly. However, in spite of these limitations, the Karplus relation has proved to be very useful qualitatively in determining the furanose ring conformations.

The coupling constants of interest are $J_{1'2'}$, $J_{2'3'}$ and $J_{3'4'}$. From the observed values of these coupling constants, the calculated values of $\phi_{1'2'}$, $\phi_{2'3'}$, $\phi_{3'4'}$ can be obtained using equations (1) and (2). Hruska *et al*[34] and Prestegard and Chan[38] postulate that the observed J_{AB} and the calculated J_{AB} values are only time-averages, that is, the ribose exists in an equilibrium between two or more buckled forms. They suggest a $C_{(2')}$ *endo* $\rightleftharpoons$ $C_{(3')}$ *endo* equilibrium for uridine.

D. Conformation of the Exocyclic CH_2OH

It has been suggested[34] that the forces in stabilizing the ribose-phosphate backbone of the polynucleosides are partly due to the rotational barriers in the five exocyclic bonds [$C_{(4')} - C_{(5')}$, $C_{(5')} - O_{(5')}$, $O_{(5')} - P$, $P - O_{(3')}$ and $O_{(3')} - C_{(3')}$.]

NMR has been applied to obtain information about the conformation of the $C_{(4')} - C_{(5')}$ bond by observing values of $J_{4'5'}$.

The three classical rotamers are shown in Figure 5. Assuming the interconversion between these rotamers is rapid, the $J_{4'5'}$ values give a weighted average of the coupling constants due to the three conformations. Their relative populations can be calculated by the following equations:-

$$(3) \qquad J_{4'5'} = P_{I} J_{gI} + P_{II} J_{gII} + P_{III} J_{tIII}$$

$$(4) \qquad J_{4'5''} = P_{I} J_{gI} + P_{II} J_{tII} = P_{III} J_{gIII}$$

where J_g and J_t are the gauche and trans coupling constants and P_I, P_{II} and P_{III} are the mole-fraction of each rotamer. If J_g and J_t remain constant for the different rotamers, equation (3) and (4) become:-

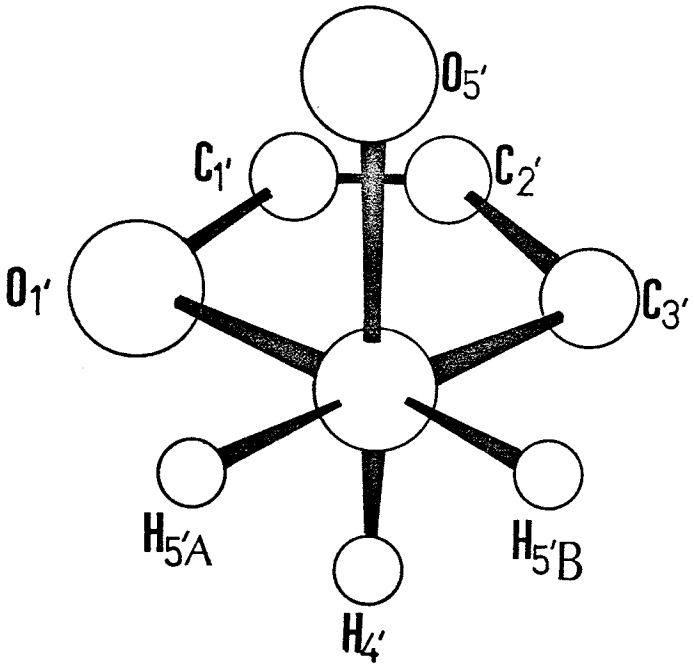
$$(5) \qquad J_{4'5'} = J_g (P_I + P_{II}) + J_t P_{III}$$

$$(6) \qquad J_{4'5''} = J_g (P_I + P_{III}) + J_t P_{II}$$

where J_g = 2.0 Hz and J_t = 10.1 Hz. The calculations of J_g and J_t employ the Karplus relation with chosen values of J° = 9.27 Hz for $0 \leq \phi \leq 90°$ and J^{180} = 10.36 Hz for $90° \leq \phi \leq 180°$ as suggested by Abraham et al.[39]

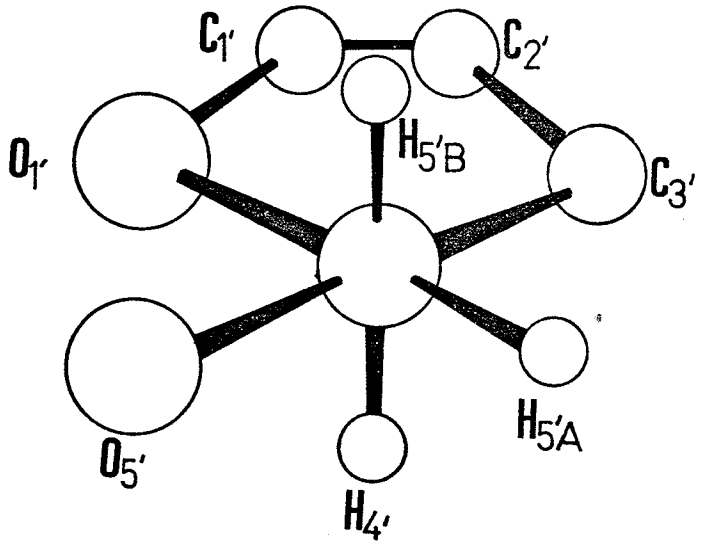
Figure 5

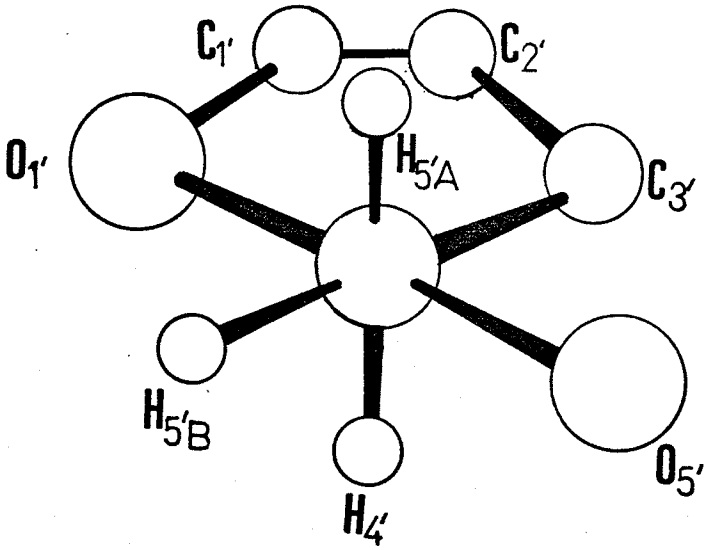
The three classical rotational isomers around the $C_{(4')} - C_{(5')}$ bond of nucleosides.



I
(gauche-gauche)

II
(gauche-trans)





III
(trans-gauche)

An oxygen-oxygen repulsion is expected to occur between $O_{(1')}$ and $O_{(5')}$, thus increasing the angle between them in rotamers I and II. In order to take this oxygen-oxygen repulsion into account, an excess 15° should be allowed when the values of J_g and J_t are calculated.[35]

According to Hruska et al[35] the gauche-gauche conformation is more favorable in both U and aU and its population decreases with increasing temperature.

CHAPTER III

EXPERIMENTAL

Experimental

The unmethylated nucleosides were obtained from Calbiochem. A set of samples of Um and Cm was a gift from Dr. D. Shugar* prepared according to the synthetic method of Janion et al.[88] Another samples of Um, Cm and Am were gifts from Dr. H. Singh** prepared by snake venom phosphodiesterase treatment of NmpN. The internal reference, DSS was obtained from E. Merck, Germany. All these were used without further purification.

Spectra of Um and Cm were obtained on the Varian HA-100 NMR spectrometer at the University of Manitoba and Varian HR-220 PMR spectrometer at the Ontario Research Foundation, Sheridan Park, Ontario. Only 100 MHz spectra of C was obtained, while 220 MHz spectra were obtained for dC, A and Am.

The concentration of each nucleoside used for the 100 MHz machine was 0.1 M DSS. The concentration for the 220 MHz machine was 0.075 M with 0.075 M DSS. The pD was adjusted to 7.0 ± 0.2 for the U and A derivatives and 8.0 ± 0.2 for the C derivatives.

The samples were freeze-dried three times with D_2O to reduce the HDO resonance. High and low temperature studies were carried out for each nucleoside using the V-4341/V-6057

* University of Warsaw

** Whiteshell Nuclear Research Establishment

variable temperature accessary with an accuracy to within ± 2.0°C.

The spectra were analysed with LAOCN3 and LAME computer programmes. The assimulated spectra were plotted by a CALCOMP 750/563 incremental pen plotter.

CHAPTER IV

RESULTS AND DISCUSSION

A. 2'-O-METHYL URIDINE VS URIDINE AND DEOXYURIDINE

I. SPECTRAL ASSIGNMENT

The experimental and computer-simulated 100 MHz spectra of Um at 28° C and 60° C are shown in Figures 6 and 7. For the sake of comparison, the 220 MHz spectra of Um at 28° C is also included. (Figure 8).

The chemical shifts and coupling constants of U, Um and dU at 28° C and 60° C as analysed from the 100 MHz spectra are recorded in Table 1. A comparison between data obtained from 100 MHz and 220 MHz for Um at 20° C is shown in Table 2. The shifts and chemical constants at 28° C analysed from the 100 and 220 MHz spectra agree well with each other within experimental errors.

The initial spectral assignments of the Um and dU were based on the 100 and 220 MHz spectra of U in aqueous solution discussed elsewhere.[40] The shifts of U are practically independent of temperature from 23° C to 80° C. Um also shows little difference in shifts (less than 1 Hz) except for $H_{(6')}$ (4.7Hz), $H_{(1')}$ (1.4Hz) and the methyl signal (1.0 Hz).

Since the spectrum of Um at high temperature has better resolution, the discussion on spectral assignment will mainly base on the spectrum at high temperature.

The doublet at 7.908 ppm is assigned to the $H_{(6)}$ proton of the pyrimidine base which has the same splitting as the $H_{(5)}$ proton signal which appears as a doublet at 5.904 ppm. The $H_{(6)}$ proton is assigned at a lower field

Figure 6

The experimental and computer-simulated 100 MHz Spectra of Um at 28° C.

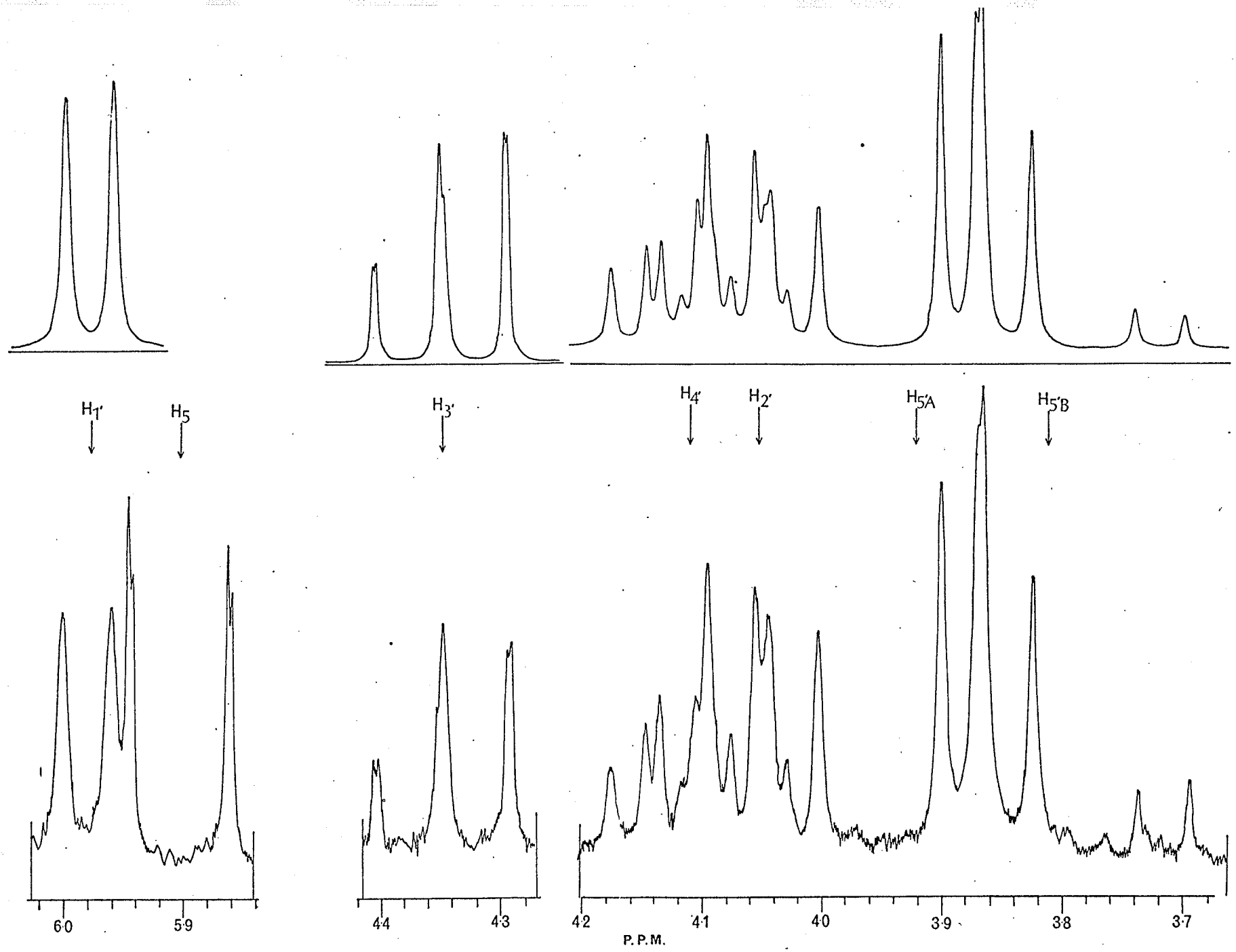

H1'
H5
H3'
H4'
H2'
H5'A
H5'B
6·0
5·9
4·4
4·3
4·2
4·1
4·0
3·9
3·8
3·7
P. P. M.

Figure 7

The experimental and Computer-simulated 100 MHz Spectra of Um at 60° C.

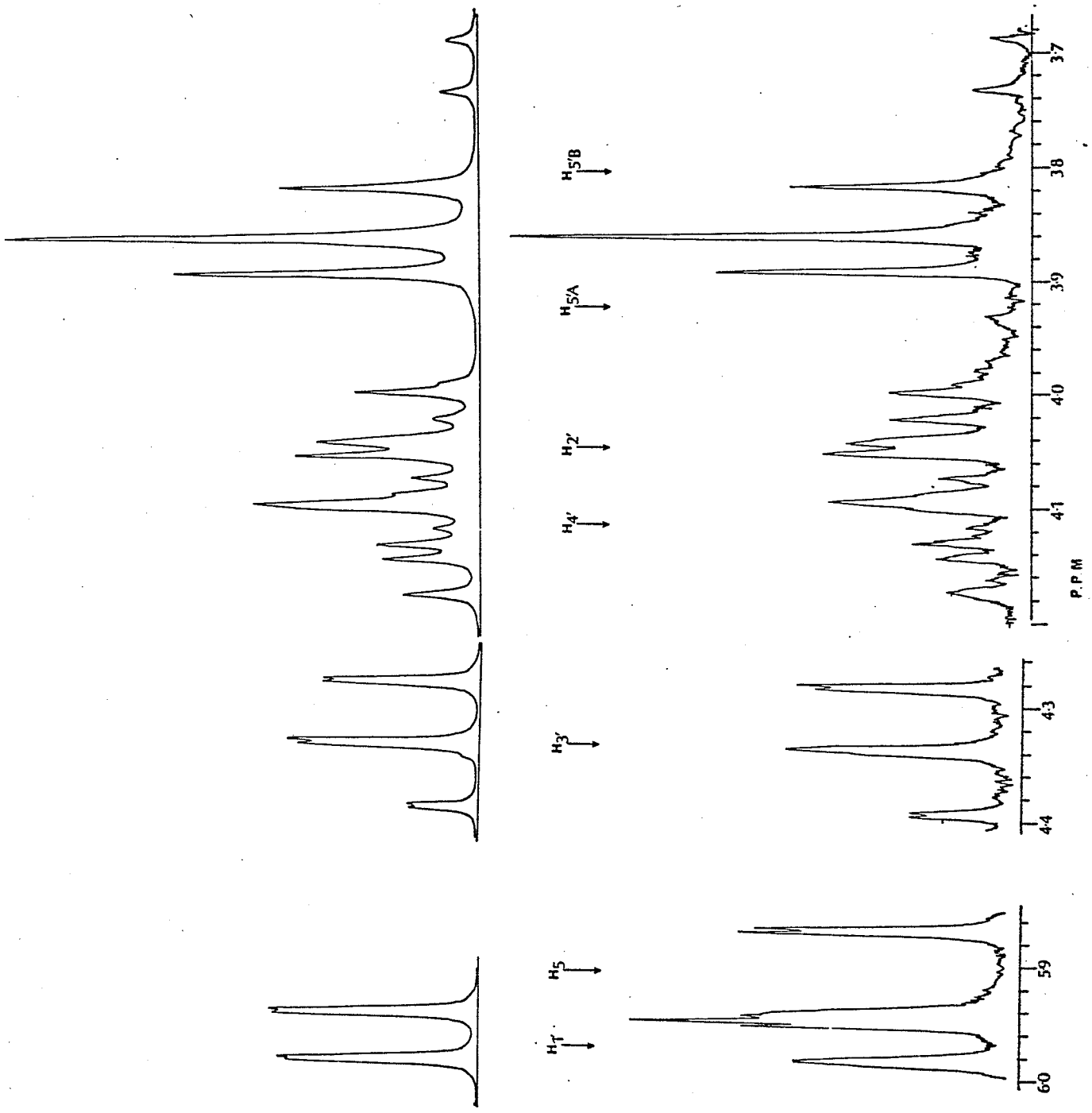

H1'
H5
H3'
H4'
H2'
H5'A
H5'B
60
5·9
4·4
4·3
4·1
4·0
3·9
3·8
3·7
P.P.M

Figure 8

The experimental and computer-simulated 220 MHz Spectra of Um at 28° C. Two spinning side bands (S.S.B.) are indicated.

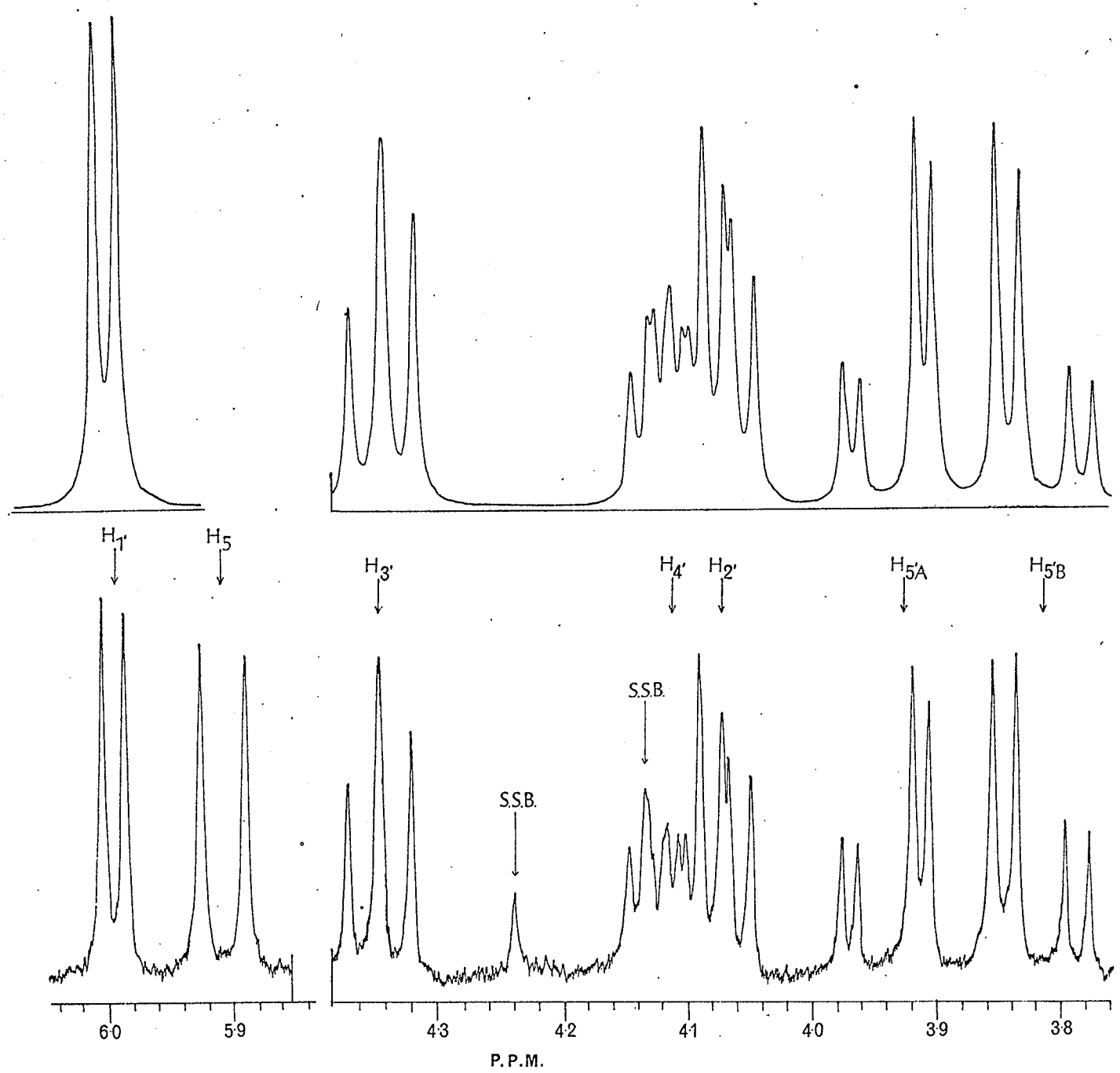

H1'
H5
H3'
H4'
H2'
H5'A
H5'B
S.S.B.
S.S.B.
6·0
5·9
4·3
4·2
4·1
4·0
3·9
3·8
P.P.M.

TABLE I

Chemical Shifts* and Coupling Constants of Um, U and dU

	Um (100 MHz)		U(100 MHz)		dU (220 MHz)**
	28° C	60° C	23° C	60° C	23° C
$H_{(6)}$	7.908	7.861	7.886	7.838	7.840
$H_{(5)}$	5.904	5.907	5.883	5.918	5.859
$H_{(1')}$	5.977	5.963	5.901	5.901	6.255
$H_{(2')}$	4.052	4.047	4.340	4.348	2' 2.349 2" 2.407
$H_{(3')}$	4.342	4.334	4.221	4.222	4.434
$H_{(4')}$	4.112	4.110	4.127	4.122	4.030
$H_{(5')}$	3.922	3.913	3.907	3.890	3.816
$H_{(5")}$	3.813	3.808	3.803	3.798	3.741
M	3.509	3.519	-	-	-
J_{56}	-	-	8.1	8.1	8.0
$J_{5'1'}$	-	0.40	-	0.35	-
$J_{1'2'}$	4.0	4.2	4.8	5.1	1'2' 6.5 1'2" 6.7
$J_{2'3'}$	5.4	5.6	5.2	5.2	2'2" -13.9 2'3' 6.8 2"3' 3.5
$J_{3'4'}$	5.9	5.6	5.4	5.1	4.0
$J_{4'5'}$	2.9	3.0	2.9	3.3	3.4
$J_{4'5"}$	4.3	4.4	4.4	4.6	5.1
$J_{5'5"}$	-12.8	-12.7	-12.7	-12.6	-12.6
$J_{1'3'}$	0.28	0.35	-	-	-

* Relative to internal DSS

** Data for dU are obtained from T. Schleich et al.[89]

TABLE 2

Chemical Shifts* and Coupling Constants of Um at 28° C from 100 MHz and 220 MHz Spectra.

	100 MHz	220 MHz
$H_{(6)}$	7.908	7.922
$H_{(5)}$	5.904	5.908
$H_{(1')}$	5.977	5.997
$H_{(2')}$	4.052	4.071
$H_{(3')}$	4.342	4.352
$H_{(4')}$	4.112	4.119
$H_{(5')}$	3.922	3.933
$H_{(5'')}$	3.813	3.820
M	3.509	3.531
$J_{1'2'}$	4.0	3.95
$J_{2'3'}$	5.4	5.45
$J_{3'4'}$	5.9	6.26
$J_{4'5'}$	2.9	2.99
$J_{4'5''}$	4.3	4.27
$J_{5'5''}$	-12.8	-13.05

* Relative to internal DSS.

than the $H_{(5)}$ because of the electro-negativity of the $N_{(1)}$ which is closer to $H_{(6)}$, thus deshielding it.

The doublet of $H_{(5)}$ is actually a doublet of doublets with a small $^{5}J_{1'5}$ splitting of 0.4 Hz. This was verified by some double resonance experiments. $^{4}J_{1'6}$ splitting is not observed because of unfavorable orientation of the bonds, that is, the path connecting $H_{(1')}$ and $H_{(6)}$ is not zig-zag.[41]

The doublet at 5.963 ppm is assigned to $H_{(1')}$. The peaks are broad with no obvious splitting. This can be explained by the fact that the $H_{(1')}$ in furanoid derivatives can couple with each of the furanose hydrogens.[42, 43]

The $H_{(2')}$ proton appears as a triplet and resonates at an unusually high field at 4.047 ppm, overlapping part of the $H_{(4')}$ and $H_{(5')}$ signals, due to methylation of the 2' position. This can be explained by studies of Narayanan and Iyer[44] on methylation on a carbon bearing a hydroxyl group, that is, methylation of a hydroxyl. They found an upfield shift of approximately 0.67 units for the methene H upon methylation of the hydroxyl of a secondary alcohol.

The $H_{(3')}$ triplet centres at 4.334 ppm. A $^{4}J_{1'3'}$ splitting of 0.35 Hz can be observed in the two outer peaks of the triplet. Again this $^{4}J_{1'3'}$ splitting was verified by double resonance experiment.

The $H_{(5')}$, $H_{(5'')}$ and $H_{(4')}$ protons form a ABX system with $H_{(5')}$ centres at 3.913 ppm, $H_{(5'')}$ at 3.809 ppm and

$H_{(4')}$ at 4.110 ppm. No attempt is made to assign the 5'-methylene hydrogens. However, Hall *et al*[45] and Lamieux[46] have attempted assignment in other instances.

The methyl protons appear as a singlet at 3.519 ppm. This assignment for the O-methyl group is based on the spectral assignments for O-methyl groups in mono-methylated D-hexoses by Gros *et al*.[47]

The $H_{(6)}$ of Um is about 2Hz downfield relative to that of U. Since the difference is small, it reflects that the sugar-base torsion angle is not much different between U and Um, otherwise it would have shown up in the shift of the $H_{(6)}$ proton as suggested by Prestegard and Chan.[48]

The effect of the 2-O-Me group on the $H_{(2')}$ is obviously diamagnetic (approximately 30 Hz) and in agreement with observations of Narayanan and Iyer[44], but paramagnetic (approximately 10 Hz) for $H_{(1')}$ and $H_{(3')}$ and negligible for $H_{(4')}$, $H_{(5')}$ and $H_{(5'')}$.

The shift of the $H_{(1')}$ proton of dU is about 0.35 ppm downfield as compared to that of U. This was explained by Gatlin and Davis[49], and T'so[50] as an electrostatic shielding of $H_{(1')}$ due to substitution of a hydroxyl group for a hydrogen at $C_{(2')}$ so that the OH group is *cis* to $H_{(1')}$. This 'CIS-OH' effect is also seen in the downfield shift $H_{(1')}$ in aU (0.246 ppm)* due to the *trans*

* relative to the corresponding proton shifts in U.

OH instead of CIS OH (data obtained from J. Dalton's master's thesis). This CIS OH shielding effect is further supported by the upfield shift of $H_{(3')}$ proton in aU* (approximately 0.1 ppm) because the 2'-OH now is CIS to $H_{(3')}$.

The upfield shift of $H_{(1')}$ in Um is 0.28 ppm relative to the corresponding proton in dU. This could be due to a shielding effect of the 'CIS-OH' group in the 2' region similar to a 'CIS-OH' effect. However, this 'CIS-OME' shielding effect on the $H_{(1')}$ proton seems to be slightly less than that of the 'CIS-OH' effect.

The $H_{(3')}$ proton in Um is shifted 0.092 ppm upfield relative to dU, showing a negligible 'trans-O-Me' shielding effect while $H_{(3')}$ in U is shifted 0.213 ppm upfield relative to dU. Thus, it seems that the shielding effect of the 2'-O-Me group on the $H_{(3')}$ is less than that of the 2'-OH group.

The shifts of $H_{(4')}$, $H_{(5')}$, and $H_{(5'')}$ of Um are virtually unaffected by 2'-O-Me group (0.02 ppm relative to U).

2. ANOMERIC CONFIGURATION

Establishment of anomeric configuration by comparison of the $H_{(1')}$ chemical shift[51] and $J_{1'2'}$[52] of the α and β anomers have met with some success.

* relative to the corresponding proton shifts in U.

In the β anomer, the $H_{(1')}$ proton is <u>trans</u> to $H_{(2')}$ and <u>cis</u> to 2'-OH. Due to the shielding effect of <u>cis</u> 2'-OH on $H_{(1')}$, we should expect an upfield shift of $H_{(1')}$ relative to that in α anomer. A survey of a variety of α and β anomers of furanoids, [52, 53, 54, 55] indicates an upfield shift of 0.22 - 0.30 ppm for the $H_{(1')}$ going from α to β anomer. Since the $H_{(1')}$ shift between U and Um is less than 0.1 ppm, we can confidently assign the Um being studied to be the β anomer.

Comparison of $J_{1'2'}$ to distinguish α and β anomers is also feasible but less satisfactory due to the limitation of the Karplus relations. Lemieux and Lineback[56] concluded that the anomeric assignment can be considered definitive only when the vicinal coupling is less than 1 Hz. However, the $J_{1'2'}$ for Um is 4.2 Hz, therefore, assignment of the anomeric configuration by Karplus relation is not attempted.

3. SUGAR-BASE TORSION ANGLE

As mentioned in the previous Chapter, the prime factors determining the anti and syn conformation are the non-bonding interaction between the ortho substituent in the base and the ribose ring. Since there is an ortho-keto group in each of Um, U and dU we can predict they should have the same favored range of sugar-base torsion angle, that is, in the anti conformation and the 2'-OMe group should have little if any effect upon the sugar-base torsion angle at the monomer level.

It is suggested recently that the 2'-OH is not likely H-bonded to the 2-keto of the pyrimidine base or even if it is H-bonded, it should not have drastic effect on the glycosidic conformation of the nucleoside[57,58,59,60]. This provides further support for the notion that the 2'OH might provide steric interference rather than chemical bonding effect. Based on this fact, we can be more confident in predicting that substituting 2'OH by 2'OMe should not alter the glycosidic conformation seriously.

According to Schweizer et al[61] and Duaga et al[62], the syn and anti conformation of pyrimidine nucleosides can be determined by making use of the 2-keto anisotropic effect upon ribose proton chemical shifts, particularly those at 2' and 3' position. They found a deshielding at $H_{(2')}$ and $H_{(3')}$ while a shielding at $H_{(1')}$, $H_{(4')}$, $H_{(5')}$, and $H_{(5'')}$ when the nucleoside is in the syn conformation.

However, in the case of Um, the effect of 2' methylation is obviously diamagnetic ($\simeq$ 0.3 ppm) for $H_{(2')}$ and paramagnetic for $H_{(1')}$ and $H_{(3')}$ and negligible for $H_{(5')}$ and $H_{(5'')}$.

Prestegard and Chan[48] has suggested that the variation in the torsion angles might be expected to result in small variation of the $H_{(6)}$ shift, because of its close proximity to the ribose moiety. The $H_{(6)}$ resonance of uridine is 0.34 ppm downfield from the corresponding resonance in uracil. This shift most likely arises from an electric field effect and/or magnetic anisotropic effect due to some group in the ribose moiety. Since $H_{(6)}$ is closest to the ethereal oxygen when in the anti conformation, this ethereal oxygen seems to be the most likely candidate. However, the $H_{(6)}$ in Um is only about 2Hz downfield from the corresponding proton in uridine, we expect the average sugar-base torsion is not seriously perturbed by the methylation in the 2' position.

As another evidence that the average N-glycosyl conformation of Um exists in the anti conformation, we can look at the long range $^5J_{1'5}$ coupling between the $H_{(5)}$ and $H_{(1')}$. $^5J_{1'5}$ can only be observed if the path from $H_{(5)}$ to $H_{(1')}$ is 'zig-zag' which can only occur when the nucleoside exists in the anti-conformation.[63] From Table I, a $^5J_{1'5}$ splitting of 0.40 Hz is observed at 60° C for Um and 0.35 Hz for U. These $^5J_{1'5}$ coupling had been verified by double resonance experiments. This observation is consistent with the former argument that the Um and U

should have the same preferred range of sugar-base torsion angle, that is, anti conformation.

4. Conformation of the Furanose Ring

As mentioned in the previous Chapter, an estimation of the ribose puckering can be obtained by Karplus treatments.[65-68] However, only the 2' endo and 3' endo cases are discussed here.[64] The $J_{1'2'}$ and $J_{3'4'}$ coupling constants provide a means of monitoring the average conformation of the ribose ring.

Smith and Jardetzky[69] have suggested twenty possible conformations of D-ribose and D-deoxyribose in nucleosides and nucleotides. Theoretical values of $J_{1'2'}$ and $J_{3'4'}$ when the ribose ring is in the 2' endo conformation are 8.6 and 0.4 Hz respectively; while $J_{1'2'}$ and $J_{3'4'}$ in the 3' endo conformations are o.4 and 8.6 cps respectively. However, the observed $J_{1'2'}$ of U, Um and dU are 4.8, 4.0 and 6.6 Hz (average of $J_{1'2'}$ and $J_{1'2''}$ for dU) respectively at 23° C; $J_{3'4'}$ of U, Um and dU are 5.4, 5.9 and 4.0 Hz at 23° C respectively. The only reasonable explanation is that the molecule is not fixed in a particular conformation but exists in a dynamic equilibrium between two or more buckled forms.[51] This equilibrium must be such that during interconversion the substituents at the 2' and 3' positions pass through the eclipsed conformation. Therefore, it appears that U, Um and dU have time average ribose conformers

intermediate between 2'-endo and 3'-endo. Comparing the $J_{1'2'}$ and $J_{3'4'}$ values of U and dU, U favors the 3' endo mode while dU is shifted towards the 2' endo conformation. From the minor changes in $J_{1'2'}$ and $J_{3'4'}$ of Um from U, it appears that a slight shift towards the $C_{3'}$ endo mode is possible. It thus appears that methylation at the 2' position has no large barrier to the pseudo rotation motion or the conformer weighings in the equilibrium blend.

5. Conformation of the Exocyclic CH_2OH Group

Using the observed values of $J_{4'5'}$ and $J_{4'5''}$, the relative populations of the three classical rotamers of Um are calculated from equations (5) and (6) and shown in Table 3.

Both U and Um show a preference of the gauche-gauche rotamer both at ambient and high temperature while dU shows only a little preference of the gauche-gauche rotamer over the gauche-trans rotamer. At 60° C, the relative populations of the rotamers seem to be only slightly different.

Comparing Um with U, it is obvious that 2'-O-methylation only has little effect on the relative populations of the exocyclic rotamers.

TABLE 3

The calculated relative populations of the three classical isomers around the $C_{(4')}-C_{(5')}$ bond of Um, U and dU.

	T°C	P_I	P_{II}	P_{III}
U	23°	0.60	0.29	0.11
	80°	0.52	0.32	0.16
Um	28°	0.61	0.28	0.11
	60°	0.58	0.30	0.12
dU	23°	0.45	0.38	0.17
	80°	0.35	0.43	0.22

6. Long Range Coupling [$^4J_{1'3'}$]

A long range coupling between $H_{(1')}$ and $H_{(3')}$($^4J_{1'3'}$) has been observed in the $H_{(3')}$ region for Um. (0.28 cps at 28° C and 0.35 cps at 60° C).

Since a $^4J_{1'3'}$ is observed in the $H_{(3')}$ region in Um, a similar splitting should be expected in U, since the vicinal J suggests similar structure. However, $^4J_{1'3'}$ is not resolved in U. A proposed explanation is that the presence of a 2'-O methyl group can make the $^4J_{1'3'}$ coupling resolvable at least in the 3' region by virtue of its shielding effect on $H_{(2')}$ and deshielding effect upon $H_{(3')}$. Thus creating a loosely coupled spin system.

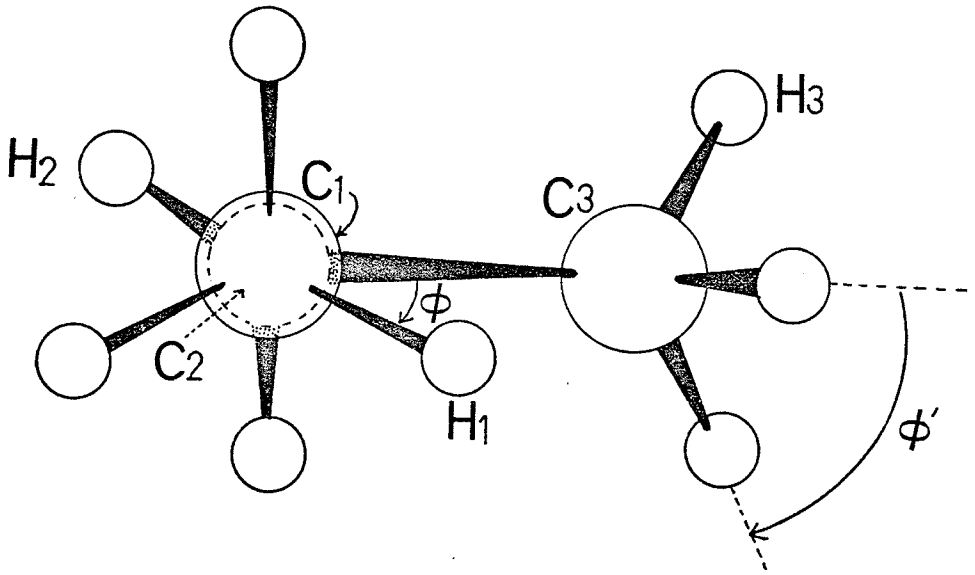
The $^4J_{1'3'}$ splitting is not observed in the $H_{(1')}$ region may be due to the fact that the $H_{(1')}$ resonance of furanose derivatives is spin-coupled to every one of the other ring protons.[70,71]

This kind of long range coupling across four bonds is common among carbohydrate derivatives.[72]

Barfield[73,74] has made a theoretical study in the four bond long range coupling by referring to a H-C-C-C-H' propane fragment. Figure 9. Calculations of $^4J_{HH'}$ has shown that the maximum absolute value occurs when the arrangement of the four bonds is in a coplanar all trans or 'W' manner, that is, $\phi = \phi = 180°$ and the sign is positive only within a narrow range of the dihedral angles near the Coplanar 'W' arrangement.

Figure 9

Specification of the dihedral angle ϕ and ϕ' (after Barfield[73]). Both angles are measured clockwise from the $C_1 - C_2 - C_3$ plane.


H2
C1
C3
H3
C2
H1
ϕ
ϕ′

This planar 'W' relationship is also seen in $^4J_{ee'}$ for a number of pyranoid carbohydrates. It has been reported by Hall[72] that $^4J_{ee'}$ (1, 3 diequatorial orientation) coupling occurs extensively and varies in magnitude from 1.2 to 1.6 Hz and all signs determined are positive. Those protons that do not have a planar 'W' arrangement have been reported to show four-bond long range coupling also; for instance, $^4J_{ea}$, (1, 3 axial-equatorial) with values of J from -0.4 to -0.7 H_z and possibly $^4J_{aa}$ (1, 3 diaxial) orientations.

It is unwise to compare the $^4J_{1'3'}$ in Um with that observed in pyranoids since their signs are not determinable. However, it is interesting to note that the $H_{(1')}$ and $H_{(3')}$ in furanose system bear a quasi-equatorial-axial relationship in both 2' endo and 3' endo puckered conformations.

7. Methoxyl Conformation

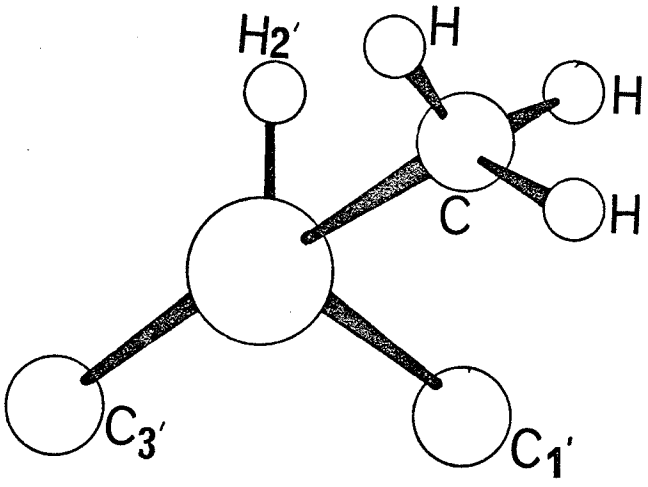
Due to its proximity to the adjacent phosphate group in polynucleotide, it is highly possible that the 2'-O Me group can play an important role in stabilizing the ribose-phosphate backbone, probably through its steric effect. In view of this, evaluation of the favored orientation of the methyl group about the $C_{(2')} - O_{(2')}$ bond is of considerable interest.

The three classical rotamers about the $C_{(2')} - O_{(2')}$ bond are shown in Figure 14. In rotamer I, the C (methyl) and $H_{(2')}$ are gauche to each other and we can see from space-filling models that there is a close contact between the 2-keto oxygen of the base and the methyl hydrogens. In rotamer II, the C (methyl) and $H_{(2')}$ are again oriented gauche but this time a close contact between the methyl and the 3'-OH group arises. In rotamer III, the C (methyl) and $H_{(2')}$ are trans oriented. However, in this trans rotamer, the movement of the methyl group and the pseudo-rotation motion of the sugar residue are severely restricted by a number of intramolecular contacts. Thus, we are inclined to think that the gauche rotamer I and II will prevail in solution.

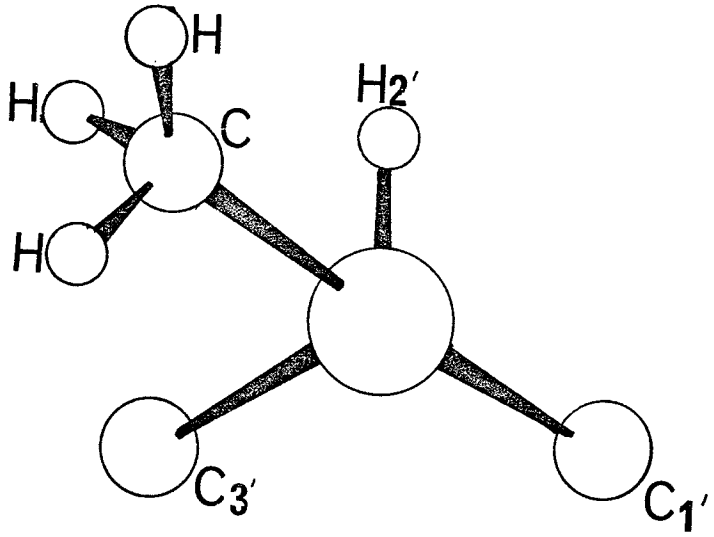
A qualitative information about the stereochemistry of the methoxy group may be obtained from the narrow line width of the methyl peak (0.6 Hz). In the trans rotamer III, a planar zig-zag path connecting $H_{(2')}$ and the

Figure 14

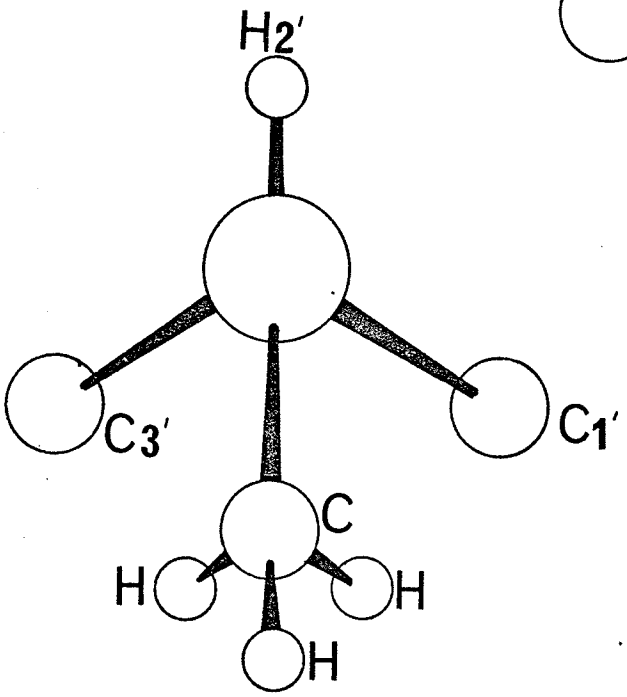
The three classically staggered conformations of 2'-methylated nucleosides about the $C_{(2')} - O_{(2')}$ bond.



I



II



III

methyl hydrogens is encountered during the course of rotation of the methyl group. Thus a four-bond coupling across the $H_{(2')}$-$C_{(2')}$-O-C(methyl)-H is not unexpected. Any $^4J_{2'm}$ coupling must be less than 0.1 Hz because of the narrow line width observed. The absence of a $^4J_{2'm}$ coupling suggests a low probability for the <u>trans</u> conformer. However, experimental data concerning the four-bond coupling across H-C-O-C-H fragments are limited and we feel no acceptable statements of stereochemical requirements for a large $^4J_{2'm}$ coupling can be made at this stage.

B. 2'-O METHYL CYTIDINE VS CYTIDINE AND DEOXYCYTIDINE

1. Spectral Assignment

The observed spectra of Cm at 28° C and 60° C are shown in Figures 10 and 11, each accompanied by a computer-simulated spectrum. The 220 MHz spectrum of Cm at 28°C is also included for the sake of comparison. (Figure 12).

The chemical shifts and coupling constants of C, Cm and dC at 25° C and 60° C as analysed from the 100 MHz spectra are shown in Table 4. Data from the 100 MHz and 220 MHz spectra are included for comparison. (Table 5). These data obtained from separate spectra agree well with each other within experimental errors.

The analysed 100 MHz and 220 MHz spectra of U in aqueous solution[40] are used as a guide for initial spectral assignments of Cm, C and dC. There is no difficulty in the assignment of the C spectrum since the 220 MHz spectra of C and U are very similar.

As in Um and U, the chemical shifts and coupling constants of Cm show little dependence on the temperature between 28° C and 60° C.

The following discussion in spectral assignments of Cm are based mainly on the high-temperature-spectrum.

The doublet at 7.831 ppm is assigned to the $H_{(6)}$ proton of the pyrimidine base. The $H_{(5)}$ proton appears as a doublet at 6.607 ppm with a same splitting as in the $H_{(6)}$ doublet.

Since the $N_{(1)}$ is closer to $H_{(6)}$ than $H_{(5)}$, the $H_{(6)}$

Figure 10

The experimental and computer-simulated 100 MHz spectra of Cm at 28° C. Two peaks are identified as EtOH due to traces of Ethanol.

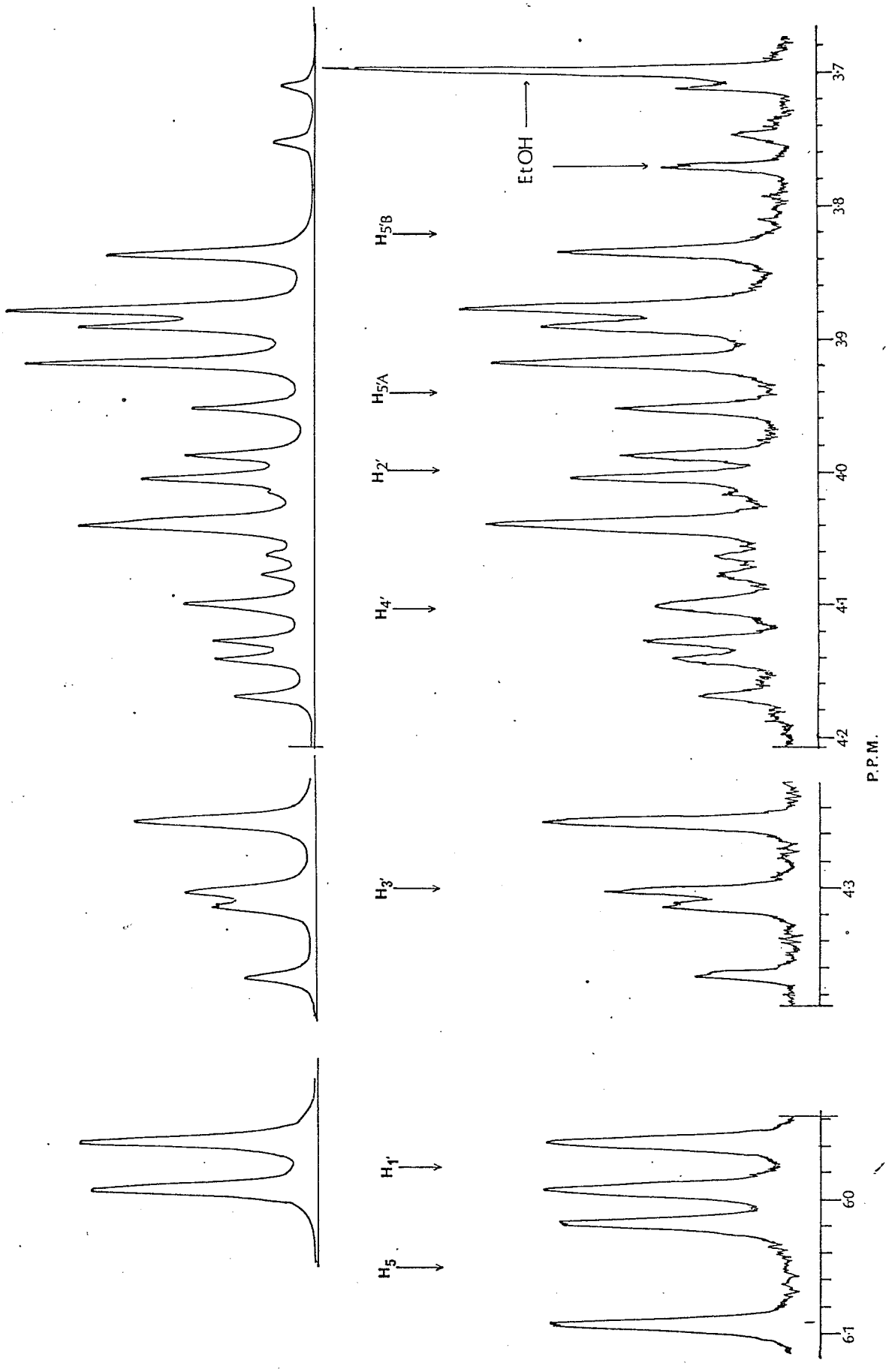

H5
H1'
H3'
H4'
H2'
H5'A
H5'B
EtOH
6·1
6·0
4·3
4·2
4·1
4·0
3·9
3·8
3·7
P.P.M.

Figure II

The experimental and computer-simulated 100 MHz spectra of Cm at 60° C. Two spinning side bands are indicated as S.S.B. Two peaks are identified as EtOH due to traces of Ethanol.

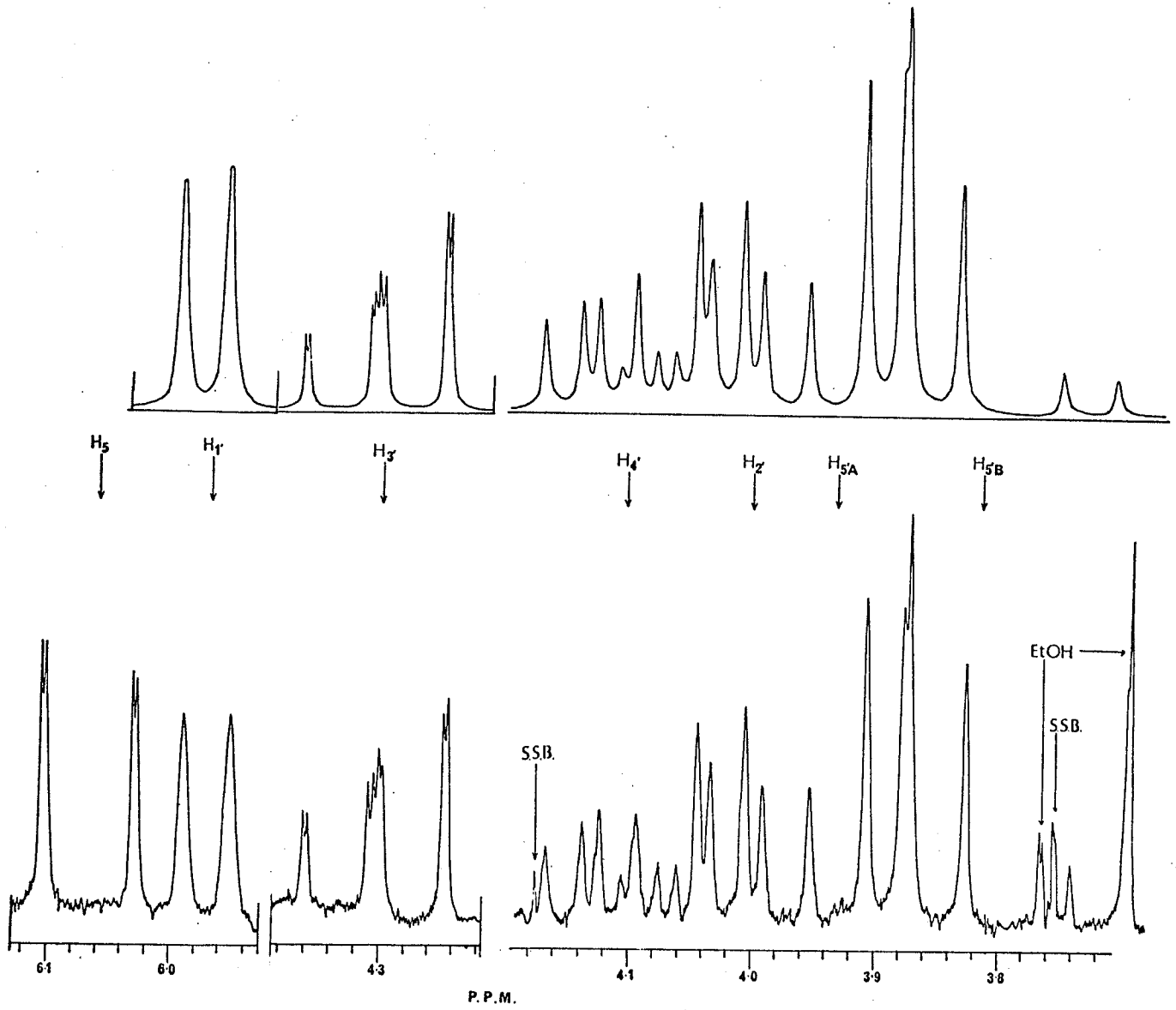

H5
H1'
H3'
H4'
H2'
H5'A
H5'B
S.S.B.
S.S.B.
EtOH
6·1
6·0
4·3
4·1
4·0
3·9
3·8
P.P.M.

Figure 12

The experimental and computer-simulated 220 MHz spectra of Cm at 28° C. Two spinning side bands (S.S.B.) are indicated.

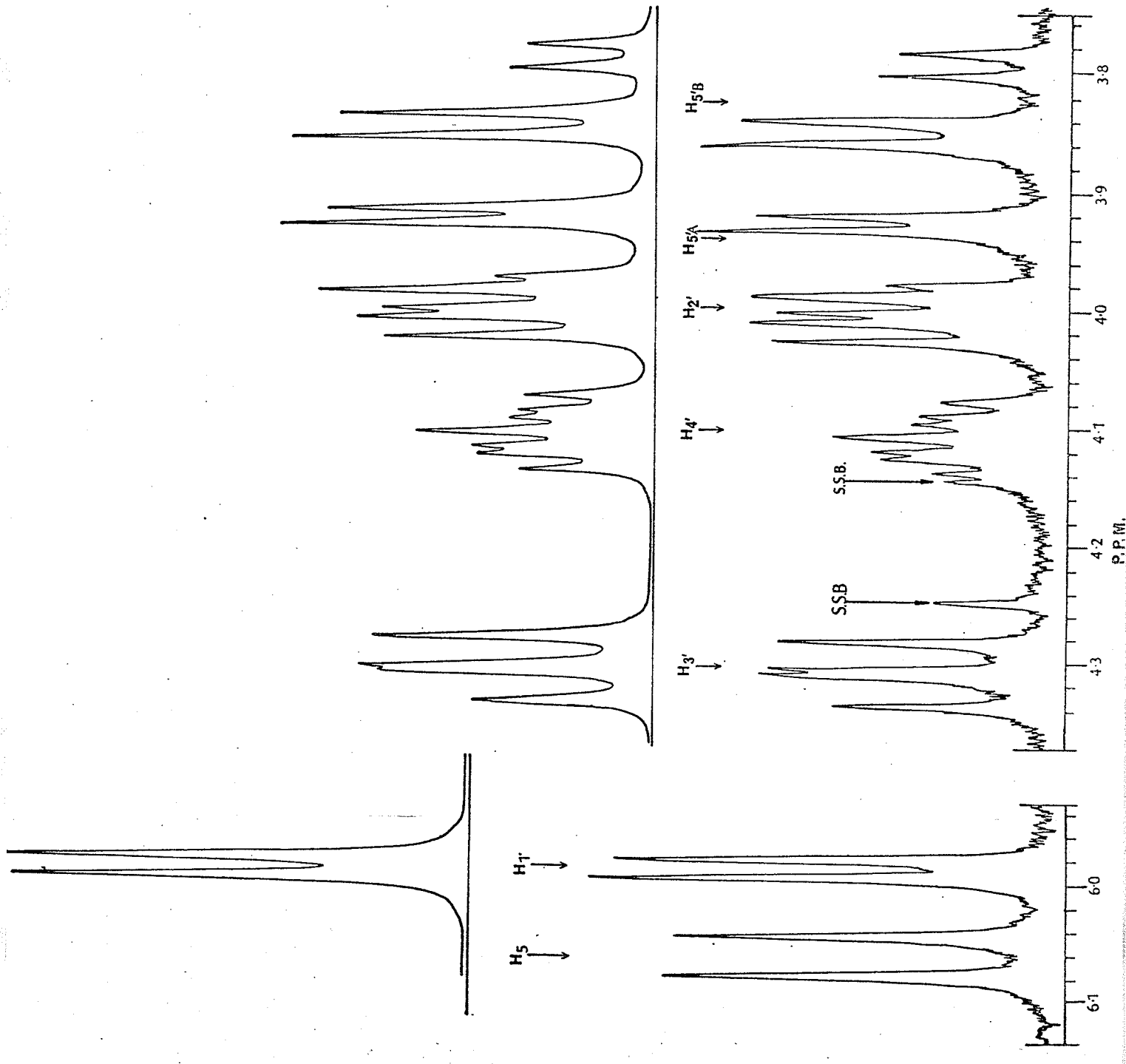

H5
H1'
H3'
H4'
H2'
H5'A
H5'B
S.S.B
S.S.B.
6·1
6·0
4·3
4·2
4·1
4·0
3·9
3·8
P.P.M.

TABLE 4

Chemical Shifts* and Coupling Constants of Cm, C and dC.

	Cm (100 MHz)		C(220 MHz)		dC (220 MHz)
	28° C	60° C	23° C	65° C	Amb.
$H_{(6)}$	7.869	7.831	7.857	7.824	-
$H_{(5)}$	6.057	6.067	6.054	6.076	-
$H_{(1')}$	5.975	5.969	5.912	5.913	6.238
$H_{(2')}$	3.997	3.999	4.315	4.324	2' 2.453 2" 2.304
$H_{(3')}$	4.301	4.295	4.215	4.220	4.448
$H_{(4')}$	4.102	4.099	4.142	4.144	4.071
$H_{(5')}$	3.938	3.925	3.942	3.933	3.852
$H_{(5")}$	3.821	3.816	3.828	3.826	3.772
M	3.535	3.524	-	-	-
J_{56}	-	-	7.6	7.6	-
$J_{51'}$	-	0.36	-	-	-
$J_{1'2'}$	3.6	3.8	4.0	4.2	1'2' 6.310 1'2" 6.650
$J^{2'3'}$	5.4	5.5	5.2	5.5	2'2" -13.94 2'3' 4.06 2"3' 6.350
$J_{3'4'}$	6.7	6.3	6.0	5.9	3.900
$J_{4'5'}$	2.8	3.0	2.8	3.1	3.650
$J_{4'5"}$	4.4	4.6	4.3	4.6	5.050
$J_{5'5"}$	-12.8	-12.9	-12.7	-12.7	-12.49
$J_{1'3'}$	0.34	0.36	-	-	-

* Relative to internal DSS, in ppm.

TABLE 5

Chemical Shifts* and Coupling Constants of Cm at 28° C from 100 MHz and 220 MHz Spectra.

	100 MHz	200 MHz
$H_{(6)}$	7.869	7.889
$H_{(5)}$	6.057	6.060
$H_{(1')}$	5.975	5.986
$H_{(2')}$	3.997	4.011
$H_{(3')}$	4.301	4.313
$H_{(4')}$	4.102	4.113
$H_{(5')}$	3.938	3.952
$H_{(5'')}$	3.821	3.829
M	3.535	3.551
$J_{1'2'}$	3.6	3.6
$J_{2'3'}$	5.4	5.4
$J_{3'4}$	6.7	6.7
$J_{4'5'}$	2.8	2.8
$J_{4'5''}$	4.4	4.4
$J_{5'5''}$	-12.8	-12.8
$J_{1'3'}$	0.34	-

* Relative to internal DSS.

therefore assigned at a lower field than $H_{(5)}$.

A $^5J_{1'5}$ splitting of 0.36 Hz is observed in the $H_{(5)}$ region thus making it a doublet of doublets. As in Um, a $^4J_{1'6}$ is not observed in the $H_{(6)}$ region due to the absence of a 'zig-zag' path[41] going from $H_{(6)}$ to $H_{(5)}$.

The doublet at 5.969 ppm is assigned to $H_{(1')}$. The $H_{(1')}$ signal is again broad, as in Um. The $H_{(2')}$ triplet appears at 3.999 ppm in agreement with the studies of Narayanan and Iyer[44] on methylation of a hydroxyl. The $H_{(3')}$ triplet centres at 4.295 ppm. A $^4J_{1'3'}$ splitting of 0.136 Hz is observed, which is verified by double resonance experiments.

As in Um, U, dU, C and dC, the $H_{(5')}$, $H_{(5")}$ and $H_{(4')}$ protons in Cm form a ABX system with $H_{(5')}$ resonances at 3.925 ppm, $H_{(5")}$ at 3.816 ppm and $H_{(4')}$ at 4.099 ppm. Again, the methyl protons appear as a sharp singlet at 3.524 ppm.

The $H_{(6)}$ of Cm resonance occurs at almost the same frequency as the H_6 in C. We can expect there should not be much difference in the sugar-base torsion angle[48] between the two.

As in Um, the effect of 2'Ome group on the $H_{(2')}$ of Cm is diamagnetic (33 Hz upfield relative to $H_{(2')}$ in C) and paramagnetic (10 Hz downfield) for $H_{(1')}$ and $H_{(3')}$ but negligible for $H_{(4')}$, $H_{(5')}$ and $H_{(5")}$.

The shift of the $H_{(1')}$ proton of C is about 0.32 ppm upfield relative to dC due to the shielding effect of the 'Cis-OH' group in the 2' region.[49,59] The $H_{(1')}$ in Cm is shifted 0.26 ppm upfield relative to that in dC; it seems that this shielding effect of an 'Cis-Ome' group is slightly less than that of an 'Cis-OH' group in the 2' region.

It is interesting to note that the $H_{(3')}$ proton in Cm is shifted only 0.14 ppm upfield relative to dC while the $H_{(3')}$ in C is shifted 0.23 ppm upfield relative to dC.

The shifts of $H_{(4')}$, $H_{(5')}$ and $H_{(5'')}$ of Cm are virtually unaffected by the 2' Ome group.

2. Anomeric Configuration

As in the case of Um, the chemical shift of the $H_{(1')}$ proton of Cm is used as a guide to establish its anomeric configuration.[51]

The chemical shift of the $H_{(1')}$ in Cm only differs from that in C by 0.06 ppm; it is apparent that the compound being studied is in the β anomer.

3. Sugar-base Torsion Angle

Determination of anti and syn conformation is approached in the same manner as for Um.

Cm, C and dC are predicted to have the same favored range of sugar-base torsion angle, that is, in the anti conformation, since in each of them there is an ortho-keto group in the base which should give the similar non-bonding interaction between the keto group and the ribose ring. Therefore, it is expected that 2' methylation should have little effect upon the sugar-base torsion angle at the monomer level.

The $H_{(6)}$ shift is only 0.7 Hz downfield from the corresponding proton in C; it again suggests that the average sugar-base torsion is not perturbed by 2' methylation.

A long range $^5J_{1'5}$ coupling of 0.36 Hz is observed in the $H_{(5)}$ region for Cm at high temperature indicating an orientation of the $H_{(1')}$ to $H_{(5)}$ is in a 'zig-zag' path and the molecule is expected to be in the anti conformation.[63] Such splittings caused by $^5J_{1'5}$ interactions are only detectable in U, Um and Cm at high temperature. A similar splitting, however, is not observed in C under comparable solution and temperature conditions.

The $H_{(5)}$ proton in this case exhibits unusually broad

resonances even at elevated temperature and pD of 8.2. At such elevated temperature and pD value, the tautomeric equilibrium between the amino and imino tautomers in C should be unimportant. This was discussed by Lee et al[75] recently. Double resonance experiments have been performed but give inconclusive results. The only reason then seems to be the presence of paramagnetic impurities in the commercial preparation of C.

4. Conformation of the Furanose Ring

The same approach as in Um to determine the conformation of the furanose ring is used here.

Referring to Table (4), the observed $J_{1'2'}$ and $J_{3'4'}$ of Cm, C and dC do not fall into any of the theoretical values of the twenty possible conformations of D-ribose and D-deoxyribose in nucleosides and nucleotides proposed by Smith and Jardetzby.[69]

As in U, Um and dU, it appears that C, Cm and dC also exist as a rapid time-average of at least two puckered forms so that during the interconversion of which the sustituents at the 2' and 3' positions pass through the eclipsed conformation.

The average $J_{1'2'}$ value of dC is 6.48 Hz and $J_{3'4'}$ is 3.90 Hz at ambient temperature. These data reflect that dC, like dU, is shifted towards the 2' endo conformation; while C and Cm favors the 3' endo conformation. If we compare the $J_{1'2'}$ and $J_{3'4'}$ of Cm with C it appears that Cm favors the 3' endo mode even more than C does.

These observations lead us to the conclusion that substitution of a 2' OH or 2'-OMe group can significantly stabilize the 3' endo conformation. However, substituting a 2' OH by 2'-OMe group does not have large effect on the barrier to the pseudorotational motion or the conformer weightings in the equilibrium blend.

5. Conformation of the Exocyclic CH_2OH Group

From equations (5) and (6), the relative populations of the three classical rotamers is calculated using observed values of $J_{4'5'}$ and $J_{4'5''}$ and shown in Table (6).

The gauche-gauche rotamer seems to be the more favorable conformer for C and Cm, while dC shows little preference of the gauche-gauche rotamer over the gauche-trans rotamer.

2'-O-Methylation does not seem to have any significant effect on the relative populations of the exo-cyclic rotamers.

6. $^4J_{1'3'}$ Long Range Coupling

A $^4J_{1'3'}$ of ≃ 0.35 Hz is observed in the 3' region for Cm both at ambient temperature and high temperature. Such $^4J_{1'3'}$ splitting is not resolvable in the 1' region due to the fact that the $H_{1'}$ is multi-coupled to every one of the other ring protons.[70,71]

Since no $^4J_{1'3'}$ splitting resoluble in C and dC, we can explain the situation by the same line of reasoning as in the case of Um, U and dU that the 2'-OMe group can

TABLE 6

The calculated relative populations of the three classical isomers around the $C_{(4')}$-$C_{(5')}$ bond of Cm, C and dC.

	T°C	P_I	P_{II}	P_{III}
C	23°	0.62	0.28	0.10
	65°	0.54	0.32	0.14
Cm	28°	0.61	0.30	0.09
	60°	0.56	0.32	0.12
dC	Amb.	0.42	0.38	0.20

create a loosely coupled spin system by shielding $H_{2'}$ and $H_{3'}$, thus making the $^{4}J_{1',3'}$ coupling resolvable at least in the 3' region.

7. Methoxyl Conformation

The line width of the 2'O methyl peak is narrow (0.7 Hz). We can follow the same line of argument as we do in Um that the probability for the trans rotamer III (Figure 14) is low due to the absence of a four-bond coupling across $H_{(2')}$-$C_{(2')}$-O-C(methyl)-H, which must be less than 0.1 Hz.

The similarity in the chemical shifts of the methyl hydrogens in Um and Cm suggests that the methyl group experiences similar environments in both molecules while the lack of any significant temperature dependence of the methyl shifts suggests that no large change $C_{(2')}$-$O_{(2')}$ rotamer populations occur in the temperature interval examined.

C. 2'-O-METHYL ADENOSINE VS ADENOSINE AND DEOXYADENOSINE

1. Spectral Assignment

220 MHz Spectra of Am were obtained at ambient temperature and shown in Figure 13, together with its computer-simulated spectrum. The chemical shifts and coupling constants are included in Table (7), together with data of A and dA.

The spectral assignments of Am are based on the 220 MHz spectrum of A.

The two base protons $H_{(2)}$ and $H_{(8)}$ are assigned at 6.795 ppm and 7.087 ppm respectively.

The $H_{(1')}$, coupled to $H_{(2')}$, appears as a doublet at 4.868 ppm. $H_{(3')}$ appears as a quartet and centres at 3.365 ppm. No $^4J_{1'3'}$ is resolvable in the 3' region keeping in mind that the spectrum being analysed is 220 MH_z.

The $H_{(2')}$ triplet is shifted ≃ 0.3 ppm upfield relative to A. This is in agreement with observation of Narayanan and Iyer[44] concerning the diamagnetic effect of an O-methyl group on the $H_{(2')}$.

The $H_{(5')}$, $H_{(5'')}$ and $H_{(4')}$ appears as an ABX subsystem and resonates at 2.668 ppm, 2.602 ppm and 3.054 ppm respectively. The methyl protons appears as a sharp singlet at 2.1909 ppm.

The $H_{(1')}$ of A is shifted ≃ 0.35 ppm upfield relative to dA. This can again be explained by the 2' Cis-OH effect of the 2' region.[49,50] The effect of the 2'Ome group seems to be paramagnetic on $H_{(1')}$ and $H_{(3')}$ but

Figure 13

The experimental and computer-simulated 220 MHz spectra of Am at ambient temperature.

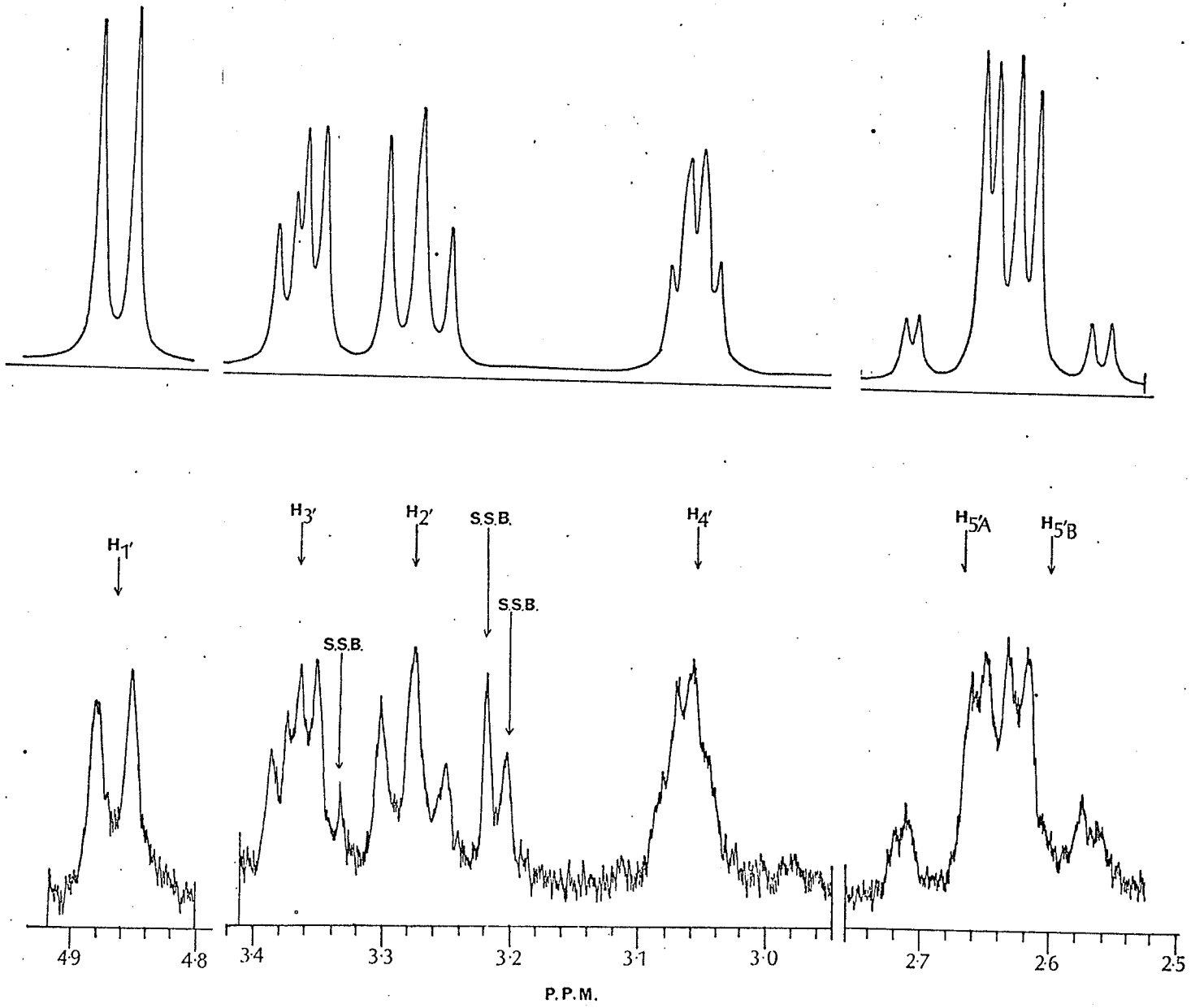

H1'
H3'
H2'
S.S.B.
S.S.B.
S.S.B.
H4'
H5'A
H5'B
4·9
4·8
3·4
3·3
3·2
3·1
3·0
2·7
2·6
2·5
P.P.M.

TABLE 7

Chemical Shifts* and Coupling Constants of Am, A and dA at Ambient Temperature, Analysed from 220 MHz Spectra.

	AM	A	dA
$H_{(2)}$	6.975	6.871	6.799
$H_{(8)}$	7.088	7.029	6.971
$H_{(1')}$	4.868	4.761	5.119
$H_{(2')}$	3.275	3.522	2' 1.509 2" 1.298
$H_{(3')}$	3.365	3.180	3.388
$H_{(4')}$	3.054	3.050	2.935
$H_{(5')}$	2.668	2.683	2.604
$H_{(5")}$	2.602	2.599	2.540
M	2.191	-	-
$J_{1'2'}$	6.093	5.930	1'2' 7.540 1'2" 6.137
$J_{2'3'}$	4.997	5.253	2'2" -13.930 2'3' 6.186 2"3' 3.092
$J_{3'4'}$	3.049	3.141	2.957
$J_{4'5'}$	2.058	2.594	3.043
$J_{4'5"}$	3.606	3.501	4.245
$J_{5'5"}$	-13.109	-12.849	-12.704

* Relative to internal DSS.

diamagnetic on $H_{(2')}$, $H_{(4')}$, $H_{(5')}$, $H_{(5'')}$ are virtually not affected by the effect of the 2'-OMe group.

2. Conformational Study of Am

Extensive conformational studies on purine nucleosides by X-ray have been carried out. Based on these X-ray data[77,78], most of the purine and pyrimidine nucleosides are found to exist in the anti conformation in solid state. However, purine conformations in aqueous state are still obscure. The energy barrier between the anti and syn conformation seems to be larger for the pyrimidine nucleosides than for the purine compounds.

ORD and C.D.[79-82] studies of purine nucleosides have created controversy about the anti and syn conformation. Ulbricht[81] proposes an anti conformation for adenosine based on the fact that adenosine has the same sign (negative) of the Cotton effect as C-8 5' cycloadenosine which has a fixed anti conformation. However, Klee and Mudd[83] suggest that adenosine may exist in a syn conformation. It seems that the question of syn and anti conformation of purine nucleosides can only be answered by results of further experimentations.

Our PMR data of Am indicate that the effect of 2'-O Methylation is paramagnetic on $H_{(1')}$ ($\simeq$ 0.11 ppm) and $H_{(3')}$ ($\simeq$ 0.18 ppm) but diamagnetic on $H_{(2')}$ ($\simeq$ 0.25 ppm).

Referring to Table (8), we can see the effect of the 2'-O Me group on $H_{(1')}$, $H_{(2')}$ and $H_{(3')}$ of A is in the same direction but with different magnitudes when compared with the effect on U and C. The difference in the shifts of $H_{(1')}$ and $H_{(3')}$ between Um and U are ≃ 0.08 ppm and ≃ 0.12 ppm respectively and those between Cm and C are ≃ 0.06 ppm and 0.09 ppm respectively.

Table (8)
Difference in Shifts of $H_{(1')}$, $H_{(2')}$ and $H_{(3')}$ in (X - Xm) pairs

	Δ(Am - A)*	Δ(Um - U)*	Δ(Cm - C)*
$H_{(1')}$	0.107 ppm	0.076 ppm	0.063 ppm
$H_{(2')}$	- 0.247	- 0.288	- 0.318
$H_{(3')}$	0.185	0.121	0.086

* at ambient temperature.

This parallel behaviour of the difference in chemical shifts of $H_{(1')}$, $H_{(2')}$ and $H_{(3')}$ in the (X - Xm) pairs suggests that the effect of 2'-O methylation is mostly electronic. The slight difference in magnitudes, however, indicates that the effect of 2'-O methylation on adenosine may have a slight redistribution of the populations of the syn and anti conformation of the molecule.

It is suggested by Jardezky[84] that adenosine exists in the $C_{2'}$ - endo conformation by observing the $J_{1'2'}$ coupling. Deoxy A[85], however, is believed to have an $C_{3'}$-exo conformation in crystalline state. From Table 7,

the $J_{1'2'}$, $J_{2'3'}$ and $J_{3'4'}$ between the A and Am pair are close enough (< 1 Hz) to suggest that any drastic effect on the furanose conformation caused by the 2'-O methylation is unlikely.

Close examination of $J_{4'5'}$ and $J_{4'5''}$ between A and Am leads us to suggest that 2'-O methylation has little effect on the population of exocyclic rotamers about the $C_{(4')}$- $C_{(5')}$ bond.

CHAPTER V

CONCLUSION

CONCLUSION

Conformational studies of 2'-O methyl uridine and cytidine are carried out using NMR technique at both ambient and high temperature (60° C). A first order analysis of 2'-O methyl adenosine is also included. The NMR data for each of these methylated nucleosides are compared with those of the corresponding normal ribosides and deoxyribosides.

The results show that Um and Cm prefer the *anti* conformation with respect to the sugar-base torsion angle. The *gauche*-*gauche* rotamer appears to be more populated than the other two exocyclic rotamers in Um and Cm. They both exist in a $C_{2'}$ *endo* $\rightleftharpoons$ $C_{3'}$ *endo* equilibrium with a slight shift towards the $C_{3'}$ *endo* mode. The conformation is independent of temperature over the range 30° C - 60° C.

The data suggest that methylation of the hydroxyl group does not have any significant effect on the conformational parameters of the nucleosides at the monomer lever, which is in agreement with *T'so et al*,[86] and F. Rottman *et al*.[87] We may conclude that the effect of 2'-O methylation on ribose conformation is consequently manifested only at the oligomer or polymer level or in less polar media.

CHAPTER VI

SUGGESTION FOR FUTURE RESEARCH

SUGGESTIONS FOR FUTURE RESEARCH

From our NMR data, it appears that 2'O methylation has little effect on the conformational parameters of nucleosides at the monomer level, at neutral pD and in aqueous solution. Thus, further research could be rewarding if attention is placed at the dimer or oligomer level.

A study of the effect of pD upon the conformational parameters of 2'-O methylated nucleosides is recommended.

A study of molecules in different nonaqueous media like DMSO, DMF and $CDCl_3$ could give valuable information about solute-solute and solute-solvent interactions.

Finally, the -OH signals can be studied if dried DMSO is used as the medium.

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