The University Of Manitoba

A CONFORMATIONAL STUDY OF SEVERAL NUCLEOSIDES IN DIMETHYL SULPHOXIDE BY PROTON MAGNETIC RESONANCE SPECTROSCOPY

by

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Special thanks to my wife, Barb, for her patience throughout.

ABSTRACT

A conformational study by proton magnetic resonance has been carried out on various β-nucleosides in dimethyl sulphoxide. The nucleoside conformations are compared in DMSO and D_2O. The ribose chemical shifts indicate that the sugar-base torsional angle (ϕ_{CN}) of pyrimidines is not significantly affected by the change in solvent; those pyrimidine nucleosides which are *anti* in D_2O appear to be *anti* in DMSO also. There is a slight increase in preference for the C_2'-*endo* (C_3'-*exo*) conformation in DMSO for the pyrimidines although the purine nucleosides do not show this change in furanose puckering. A correlation between the ribose puckering and the rotation of the exocyclic C_4'-C_5' bond has been demonstrated in D_2O[50] and is shown here to exist in dimethyl sulphoxide also. For several nucleosides the hydroxyl proton chemical shifts and coupling constants are reported. Although the OH_5' group of pyrimidines may be freely rotating the same group of purine nucleosides is apparently hindered in DMSO.

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CHAPTER I

Introduction

A. Nucleoside Structure, Function And Occurrence

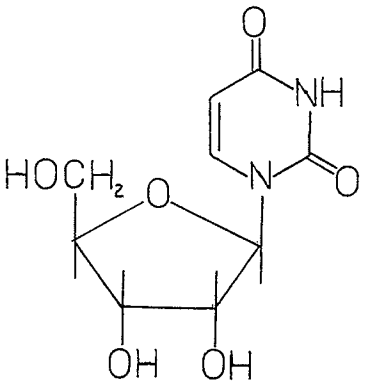
Nucleosides are N-glycosyl derivatives of the purine or pyrimidine bases (Figure 1). Ribonucleosides and 2'-deoxyribonucleosides contain D-ribose and 2'-deoxy-D-ribose, respectively, as the sugar component. The predominant naturally occurring nucleosides are derivatives of the pyrimidines uracil, thymine and cytosine and the purines adenine and guanine. The glycosyl linkage is β from N_1 of pyrimidines and N_9 of purines to the C_1' of the furanose. These nucleosides are the monomeric units of nucleic acids (ribonucleosides in RNA, 2'-deoxyribonucleosides in DNA) which are joined by a 3' to 5' phosphodiester linkage. In addition to the 'common' nucleosides, at least thirty five 'minor' nucleosides have been isolated from nucleic acids, in particular tRNA. Many of these are methyl derivatives of the major nucleosides. Other nucleosides participate as coenzymes in energy transformations and functional-group transfer reactions.

Many antibiotics are structurally related to the purine and pyrimidine nucleosides. Cordycepin (3'-deoxyadenosine) was the first of over thirty nucleoside antibiotics to be isolated[1]. The role of these nucleosides as antibiotics has resulted in an increased interest in the laboratory synthesis of modified nucleosides.

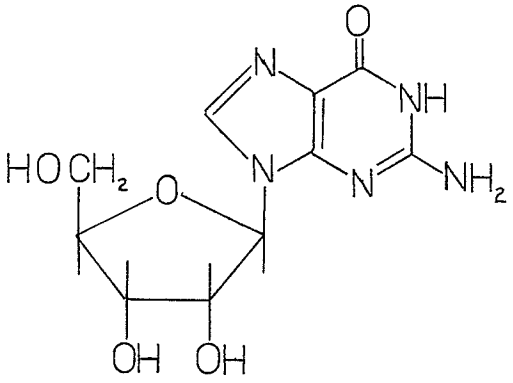
Figure 1

The eight common nucleosides of nucleic acids.

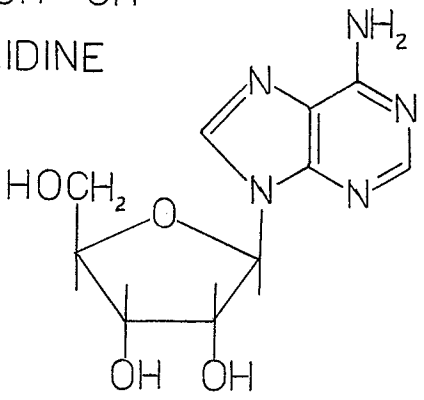
RIBONUCLEOSIDES



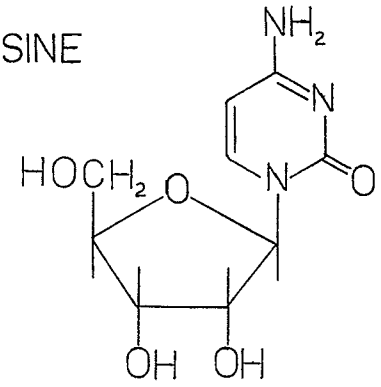
URIDINE



GUANOSINE

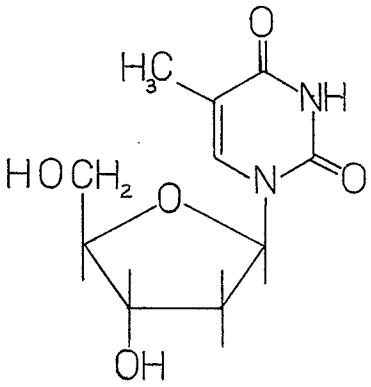


ADENOSINE

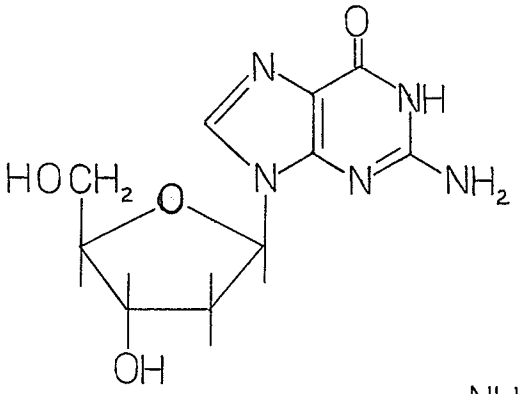


CYTIDINE

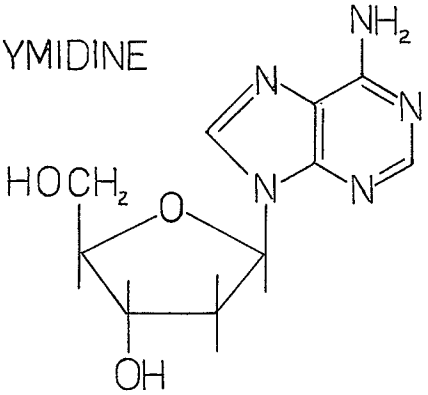
2'-DEOXYRIBONUCLEOSIDES



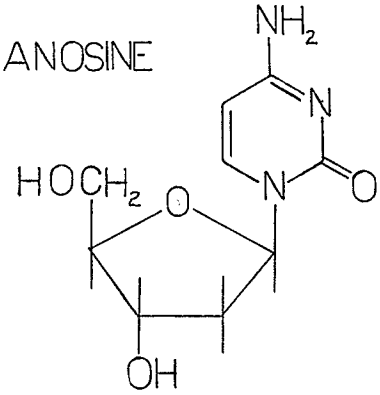
THYMIDINE



2'-DEOXYGUANOSINE



2'-DEOXYADENOSINE



2'-DEOXYCYTIDINE

B. Nucleoside Conformation

It is generally accepted that the three dimensional structure of nucleic acids is dependent upon the component nucleotides. The crystallographic data on these monomers are used extensively as guidelines for solving structural problems concerning nucleic acids. Similarly, thorough conformational studies in solution at the monomer level are a logical first step to understanding the conformation of polynucleotides and nucleic acids in solution.

1. Features Of Nucleoside Conformation

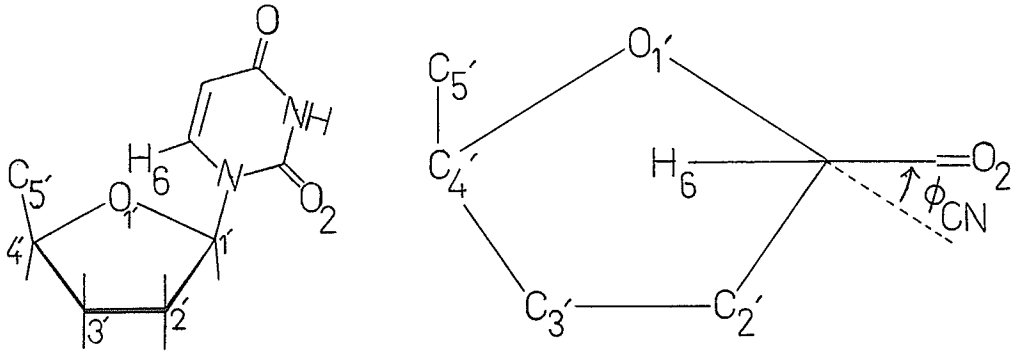
To simplify a discussion of nucleoside conformations it is useful to define certain structural parameters.

a. Sugar-Base Torsional Angle (ϕ_{CN})

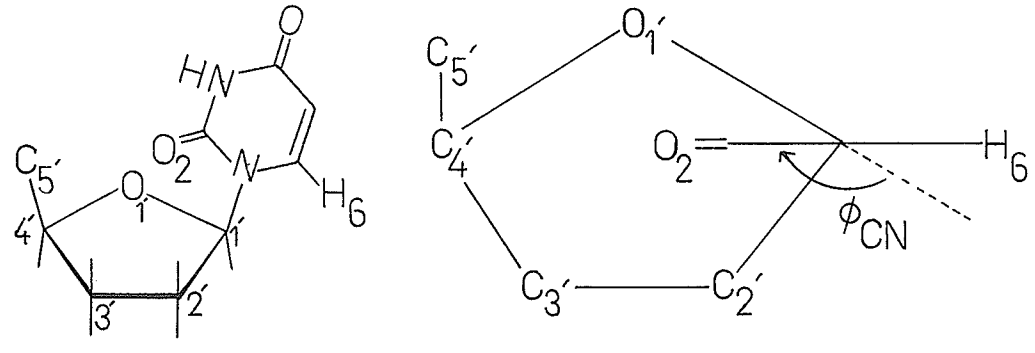
Donahue and Trueblood[2] have defined the sugar-base torsional angle (ϕ_{CN}) as the dihedral angle between the planes formed by O_1'-C_1'-N_1 and C_1'-N_1-C_2 (Figure 2). This angle is zero when the N_1-C_2 bond is *trans* to the C_1'-O_1' bond. Two sets of ϕ_{CN} values result in relative energy minima; ca. -30° ±45° for the *anti* conformation and ca. +150° ±45° for the *syn* conformation. X-Ray[3] and solution[4] studies have shown that the majority of nucleosides prefer the *anti* conformation; this conformation has also been observed in nucleic acids[5]. Kapuler *et al*[6] found that enzymatic polynucleotide synthesis may not proceed if the di- or triphosphate substrates do not have

Figure 2

The sugar-base torsional angle (ϕ_{CN}), with positive values measured in a clockwise direction.


O
NH
H6
N
O2
C5′
O1′
4′
1′
3′
2′
C5′
O1′
C4′
H6
O2
ϕCN
C3′
C2′

ANTI


O
HN
O2
N
H6
C5′
O1′
4′
1′
3′
2′
C5′
O1′
C4′
O2
H6
ϕCN
C3′
C2′

SYN

the anti conformation.

The barrier to interconversion of the syn and anti conformations in pyrimidine nucleosides results from steric interaction between the C_2 keto oxygen or the C_6 proton and the furanose ring. The steric interaction for purine nucleosides is between N_3 of the base and the furanose.

b. The Exocyclic CH_2OH Group

The three conformations in which all substituents are staggered are shown in Figure 3. NMR and X-ray studies have found that the gauche-gauche (gg) conformation is most often observed for nucleosides and nucleotides.

c. The Furanose Ring

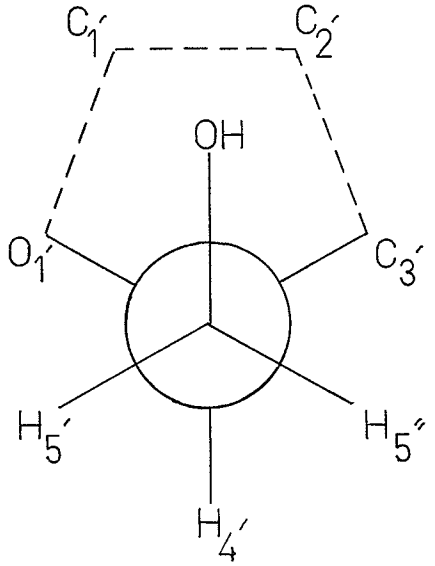
To alleviate the steric interaction of substituents on adjacent carbon atoms, the furanose ring assumes a puckered conformation in which one or two atoms deviate from the plane defined by the remaining atoms (Figure 4). The out-of-plane carbon may deviate to the same side as the C_5' atom or to the opposite side. This corresponds to the endo and exo conformations, respectively.

d. The Purine And Pyrimidine Bases

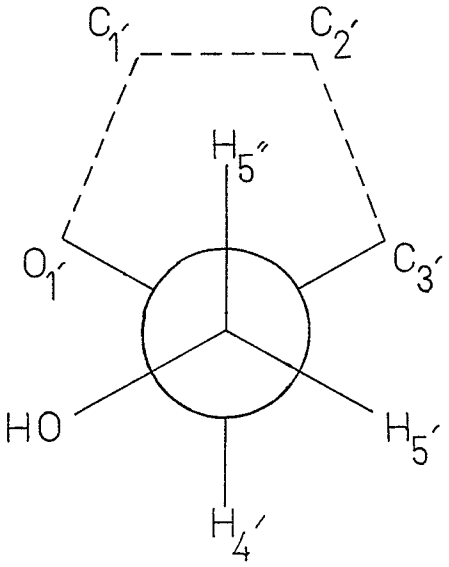
X-Ray analyses have shown that the heterocyclic bases are almost planar. Minor exceptions include various substituted pyrimidines[7] and 5,6-dihydrouracil[8].

Figure 3

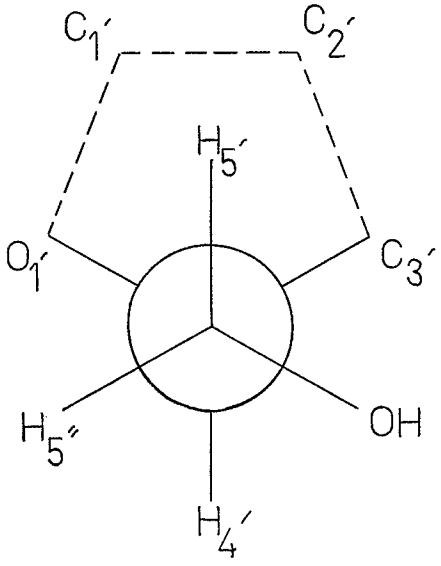
The three staggered conformations about the C_4'-C_5' exocyclic bond.



GAUCHE-GAUCHE (gg)



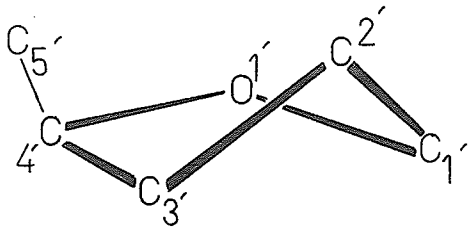
GAUCHE-TRANS (gt)



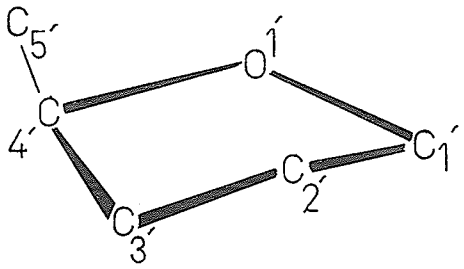
TRANS-GAUCHE (tg)

Figure 4

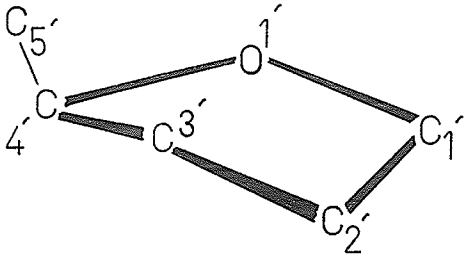
The C_2' and C_3' puckered conformations of the furanose ring.



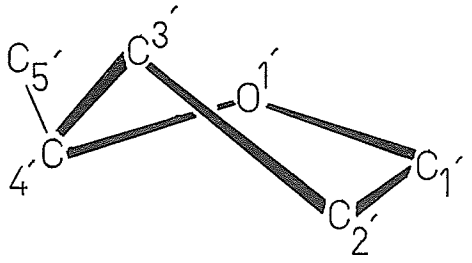
$C_{2'}$-ENDO



$C_{3'}$-EXO



$C_{2'}$-EXO



$C_{3'}$-ENDO

2. Physicochemical Methods Of Study

a. X-Ray Diffraction

This technique has proven extremely valuable for the determination of the complete conformation of nucleosides in the solid state. The majority of both pyrimidine and purine nucleosides have been shown to favor the anti conformation[3], however a few, including 4-thiouridine[9] and deoxyguanosine (in 5-bromocytidine·deoxyguanosine complex)[10], have been found in the syn conformation. Studies by Sundaralingam[3] on the puckering of the furanose have revealed that the C_2' and C_3' endo and exo conformations are most often observed. Of the three staggered conformations about the exocyclic C_4'-C_5' bond, most nucleosides have shown preference for the gauche-gauche conformer, although the gauche-trans (gt) and trans-gauche (tg) have also been found.

b. Optical Rotatory Dispersion (ORD) And Circular Dichroism (CD)

Conformational studies of nucleosides in solution have proven more difficult than those done in the solid state. ORD-CD studies in D_2O have yielded information about the sugar-base torsional angle (ϕ_{CN}) which indicates that most pyrimidines are anti[11], as has been found in the solid by X-ray. Miles et al[12] have provided CD evidence that pyrimidines in the syn conformation include those bearing a bulky substituent at the C_6 position of the base.

The ORD-CD results for purines are less conclusive, although in general, the anti conformation is suggested. The CD curves of purines show marked changes upon substitution of a bulky group at the C_8 position of the base; this has been interpreted as resulting from a change in ϕ_{CN} from anti to syn[13]. Miles et al have demonstrated that ϕ_{CN} of both pyrimidines[14] and purines[15] may be significantly affected by various solvents and pH. These authors have concluded that in solvents unable to form strong hydrogen bonds, the syn conformation may be stabilized by the formation of intramolecular hydrogen bonds (from the C_2-keto to the OH_5' in pyrimidines and from the N_3 to the OH_5' in purines).

c. Nuclear Magnetic Resonance (NMR)

NMR studies have provided valuable information concerning the puckering of the ribose in solution. Hruska et al[16,17] have reported the complete analyses of several nucleosides in D_2O and concluded that the ribose coupling constants can not be adequately explained by any one conformer. Rather, a C_2'-endo,C_3'-exo $\leftrightarrow$ C_3'-endo,C_2'-exo type of rapid conversion best explains the results (vide infra). Rotation about the exocyclic C_4'-C_5' bond is expected to be rapid in solution. However, the evidence suggests that most nucleosides show a time-averaged preference for the gauche-gauche rotamer. The NMR and CD results are in agreement that most pyrimidines are anti unless a bulky substituent is present at the C_6 position of the component base[18]. Information on the sugar-base torsional

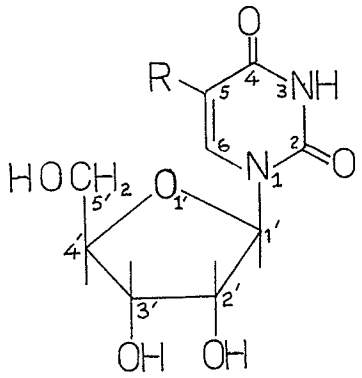
angle has been difficult to obtain in the case of purines, although the 5'-purine nucleotides appear to be anti in D_2O[20].

The present NMR study was carried out in dimethyl sulphoxide-d6 (DMSO-d6) as an initial step to determine the extent to which the solvent may influence the nucleoside conformation. The majority of nucleoside conformational studies by NMR have employed the solvent D_2O. However, ORD-CD results of Miles et al[14,15] indicate that the sugar-base torsional angle of certain nucleosides is dependent upon the solvent. Iball et al[21] found that 5-bromouridine crystallized from dimethyl sulphoxide showed slight differences from that crystallized from D_2O, when analyzed by X-ray diffraction. Thermodynamic[22] and NMR[23] evidence indicates that certain nucleosides tend to self-associate in aqueous solution, probably by specific association of the hydrophobic bases. Therefore, various conformational differences among nucleosides in D_2O may simply be a manifestation of increased or decreased self-association. Dimethyl sulphoxide is one of the few less polar solvents capable of dissolving most nucleosides. This solvent also slows the proton exchange process so that protons on oxygen and nitrogen will often give well resolved multiplets, resulting from coupling to protons on adjacent carbon atoms[24]; this has led to the extensive use of DMSO as solvent for the conformational study of carbohydrates[25] and alcohols[26]. The nucleosides examined

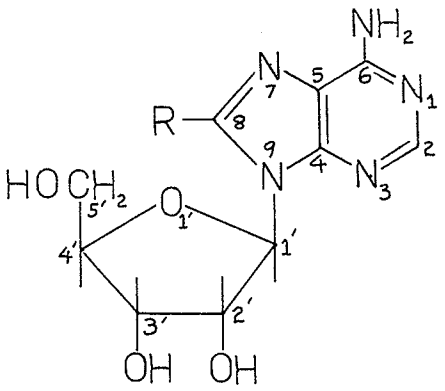
in dimethyl sulphoxide are shown in Figure 5. In the following chapters, the conformations of these nucleosides in dimethyl sulphoxide are discussed and compared with the conformations reported in aqueous solution.

Figure 5

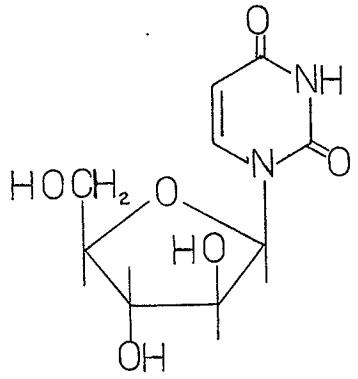
The nucleosides examined in dimethyl sulphoxide.



1. URIDINE (R = H)
2. 5-IODOURIDINE (R = I)
3. 5-COOEtURIDINE (R = COOEt)



4. ADENOSINE (R = H)
5. 8-BROMOADENOSINE (R = Br)



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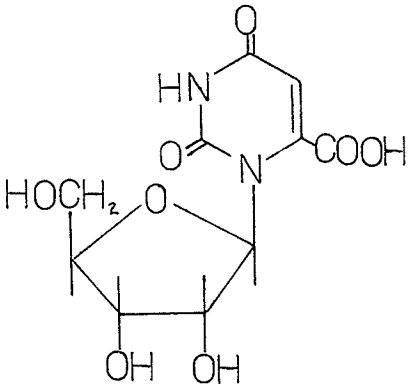
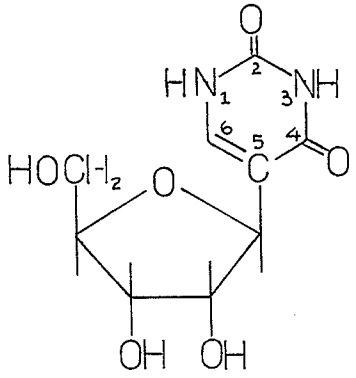
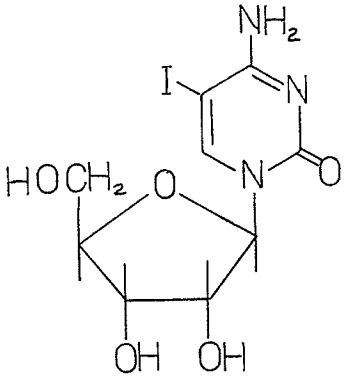


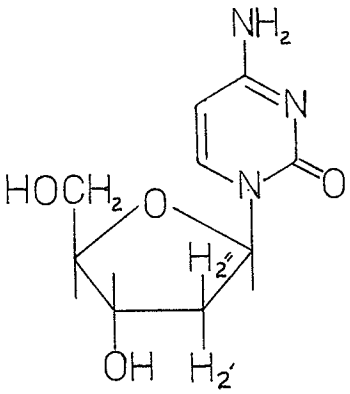
7. OROTIDINE



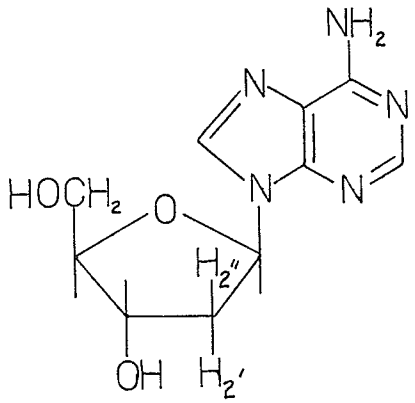
8. PSEUDOURIDINE



9. 5-IODOCYTIDINE



10. 2′-DEOXYCYTIDINE



11. 2-DEOXYADENOSINE

CHAPTER II

Experimental

The nucleosides were obtained from Calbiochem, Aldrich Chemicals and Sigma Chemicals; all compounds were Grade A. Dimethyl sulphoxide-d6 (99.5% D) was purchased from Stohler Isotope Chemical Co. and stored over molecular sieve (Linde 3A). All materials were used without further purification. Two samples of each nucleoside were prepared at a concentration of 0.15M. One sample of each nucleoside was lyophillized from D_2O, to replace the exchangeable protons with deuterons, and redissolved in DMSO-d6. A second sample was heated under vacuum at 100°C. for several hours, to drive off residual water, and then dissolved in DMSO-d6 in a dry nitrogen atmosphere. The spectra of adenosine (A), 8-bromoadenosine (8-BrA), 2'-deoxyadenosine (dA) and 2'-deoxycytidine (dC) were recorded on a Varian HA-100D proton magnetic resonance spectrometer at the University of Manitoba. Spectra of uridine (U), 5-iodouridine (5IU), arabinouridine (AU), β-pseudouridine (β-ψU), 5-iodocytidine (5IC) and orotidine (O) were recorded on a Varian HR-220 proton magnetic resonance spectrometer at the Ontario Research Foundation, Sheridan Park, Ontario; this was necessary due to the complexity of the 100 MHz. spectra. All spectra were recorded at ambient temperature with chemical shifts reported relative to internal tetramethylsilane (TMS). The computer simulations were carried out using LAME[53] on an IBM 360/65 computer and CALCOMP 750/563 incremental plotter at the University of Manitoba.

CHAPTER III

Spectral Assignment

The assignment of chemical shifts (ν) and coupling constants (J) will be discussed briefly for adenosine, 2'-deoxyadenosine and 8-bromoadenosine. The remaining nucleosides reported in this study have been analyzed in a similar manner and the results are listed in Table I.

A. Adenosine

1. Nonexchangeable Protons

The observed 100 MHz. spectrum and computer simulation of adenosine in DMSO-d6 are shown in Figure 6. The initial assignment of the furanose proton chemical shifts was made by comparison with a partial analysis of adenosine in 40% benzene/dimethyl sulphoxide at 60 MHz. carried out by Davies and Danyluk[28]. Their assignment was confirmed in this laboratory by double resonance experiments.

The anomeric proton, H_1', is found at 5.89 ppm which is downfield from the remaining nonexchangeable furanose protons due to the inductive effects of the base and the ring oxygen. The multiplets resulting from the H_2', H_3' and H_4' protons are well separated at 100 MHz. so that no virtual coupling is observed. The methylene protons, H_5' and H_5'', give rise to the AB part of an ABX subspectrum (where H_4' = X). Although the high field satellite of the H_5'' proton is obscured by a side band of water in this sample, the value of $J_{4'5''}$ was ascertained from the H_4' resonance and also from the spectrum of

Table I

a. Chemical Shifts of the Ribonucleosides in DMSO-d6 at Ambient Temp.

b. Coupling Constants of the Ribonucleosides in DMSO-d6 at Ambient Temp.

c. Chemical Shifts and Coupling Constants of the 2'-Deoxyribonucleosides in DMSO-d6 at Ambient Temp.

Table Ia

chem.† shift	Adenosine	8-Bromo-adenosine	Uridine	5-Iodo-uridine	5-COOEt uridine	β-Pseudo uridine	5-Iodo-cytidine	Arabino uridine	Orotidine
ν_1'	5.891	5.849	5.784	5.708	5.787	4.470	5.709	5.993	5.558
ν_2'	4.621	5.096	4.027	3.960	4.070	3.940	3.87 ††	4.011	4.384
ν_3'	4.162	4.210	3.965	3.907	3.994	3.890	3.87 ††	3.911	4.014
ν_4'	3.982	3.993	3.852	3.798	3.916	3.730	3.777	3.751	3.587
ν_5'	3.688	3.682	3.620	3.606	3.710	3.625	3.630	3.622	3.531
ν_5''	3.570	3.531	3.551	3.496	3.593	3.475	3.484	3.588	3.363
pyrimidines:									
ν_5	-----	-----	5.639	-----	-----	ν_1=10.83 ν_3=11.06	-----	5.583	5.235
ν_6	-----	-----	7.871	8.410	8.914	7.513	8.376	7.649	-----
purines:									
ν_2	8.168	8.148	-----	-----	-----	-----	-----	-----	-----
ν_8	8.360	-----	-----	-----	-----	-----	-----	-----	-----
Exchangeable:									
ν_{02}'	5.405	5.419	5.391	5.355 ††	-----	4.842 ††	5.302 ††	5.590	-----
ν_{03}'	5.145	5.188	5.098	5.010 ††	-----	4.624 ††	4.928 ††	5.464	-----
ν_{05}'	5.381	5.458	5.109	5.205	-----	4.715	5.165	5.051	-----
ν	C_6-amino 7.303	C_6-amino 7.577	-----	-----	-----	-----	-----	N_3imino 11.20	-----

† ppm downfield from internal TMS (in DMSO-d6).

†† the close proximity of the H_2' and H_3' shifts prevented an absolute assignment.

Table Ib

(Hz.)	Adenosine	8-Bromo-adenosine	Uridine	5-Iodo-uridine	5-COOEt uridine	β-Psuedo uridine	5-Iodo-cytidine	Arabino uridine	Orotidine
$J_{1'2'}$	6.12	6.66	5.50	4.60	4.02	4.40	3.50	4.54	3.60
$J_{2'3'}$	4.87	5.16	5.23	5.10	4.74	4.95	5.10	3.40	6.05
$J_{3'4'}$	3.22	2.33	3.57	4.40	5.14	5.64	5.30	3.64	5.95
$J_{4'5'}$	3.78	3.97	3.35	2.60	2.59	3.09	2.50	4.4]	3.15
$J_{4'5''}$	3.39	4.05	3.22	2.50	2.35	3.50	2.45	5.58	6.20
$J_{5'5''}$	-12.03	-12.21	-12.04	-12.00	-11.89	-11.90	-12.00	-11.75	-11.80
pyrimidines:			J_{56}=8.2			$J_{1'6}$=0.7 J_{16}=5.60		J_{35}=1.8 J_{56}=8.0	
hydroxyls:									
$J_{2'02'}$	6.17	6.26	5.45	5.00†	-----	4.80†	5.25†	5.05	-----
$J_{3'03'}$	4.61	4.47	4.79	4.50†	-----	5.60†	5.00†	4.16	-----
$J_{5'05'}$	4.69	3.81	4.95	~4.65	-----	4.75	~4.75	5.39	-----
$J_{5''05'}$	6.96	8.52	4.90	~4.65	-----	6.20	~4.75	5.51	-----

† only approximate values due to virtual coupling

Table Ic

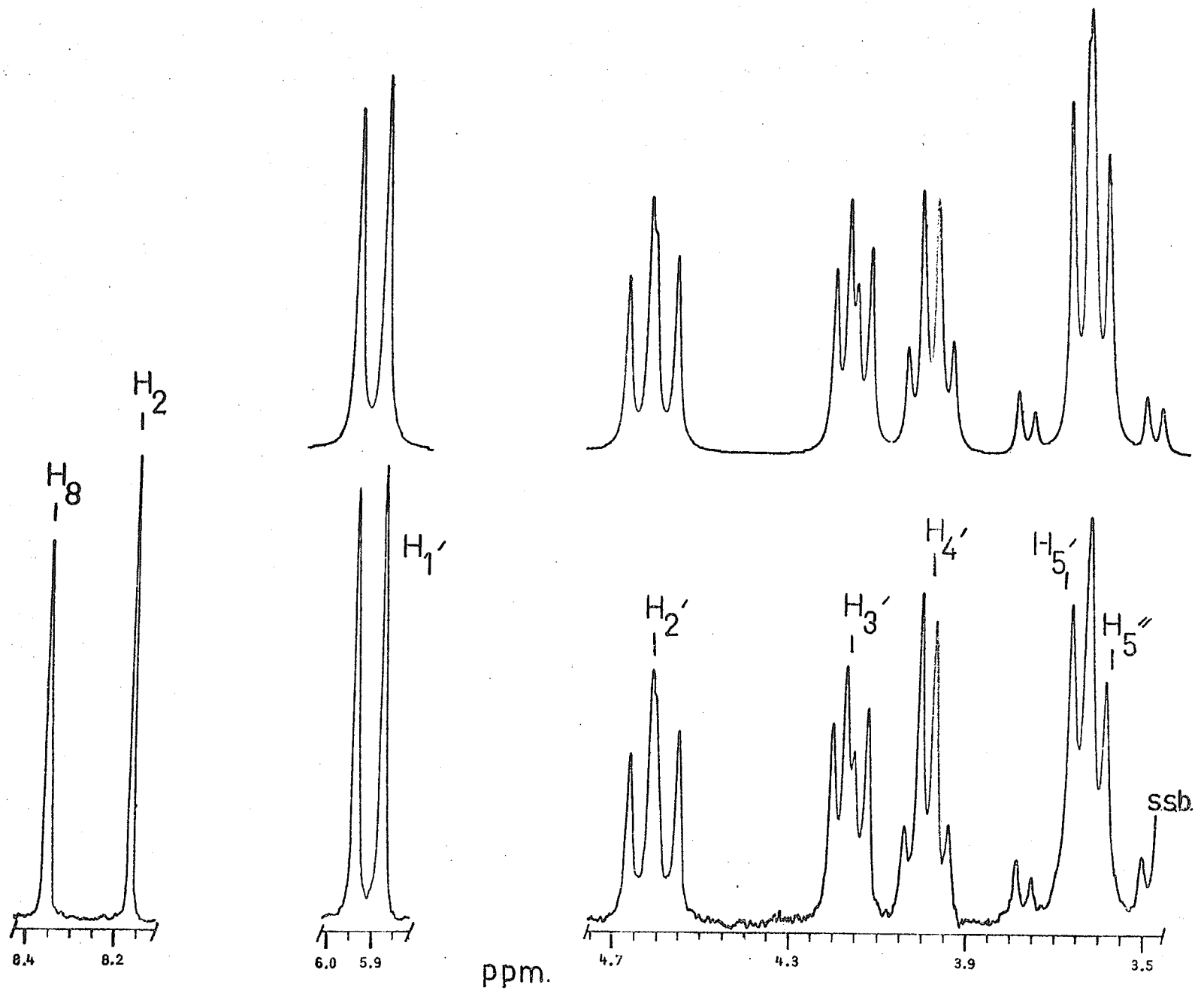
	2'-Deoxy-adenosine	2'-Deoxy-cytidine
$\nu_{1'}$ †	6.354	6.156
$\nu_{2'}$	2.277	2.100
$\nu_{2''}$	2.733	1.930
$\nu_{3'}$	4.418	4.201
$\nu_{4'}$	3.896	3.771
$\nu_{5'}$	3.626	3.566
$\nu_{5''}$	3.531	3.523
ν_5	-----	5.721
ν_6	-----	7.786
ν_2	8.138	-----
ν_8	8.328	-----
ν_{NH_2}	7.279	-----
$\nu_{O3'}$	5.272	-----
$\nu_{O5'}$	5.202	-----

(Hz.)	2'-Deoxy-adenosine	2'-Deoxy-cytidine
$J_{1'2'}$	6.13	6.00
$J_{1'2''}$	7.74	7.30
$J_{2'2''}$	-13.29	-13.00
$J_{2'3'}$	2.86	3.20
$J_{2''3'}$	5.71	5.85
$J_{3'4'}$	2.49	3.20
$J_{4'5'}$	4.34	3.75
$J_{4'5''}$	3.95	3.75
$J_{5'5''}$	-11.80	-13.00
J_{56}	-----	7.50
$J_{3'O3'}$	4.00	----
$J_{5'O5'}$	4.72	----
$J_{5''O5'}$	6.49	----

† chemical shifts in ppm downfield from internal TMS. in DMSO.

Figure 6

The observed 100 MHz. spectrum and computer simulation of adenosine in DMSO-d6, showing all nonexchangeable protons.


H2
H8
H1′
H2′
H3′
H4′
H5′
H5″
ssb
8.4
8.2
6.0
5.9
4.7
4.3
3.9
3.5
ppm.

adenosine including the exchangeable protons (see Figure 7). Remin and Shugar[27] have recently noted that the phosphate group of 3'-nucleotides exhibits a selective influence upon the H_5' and H_5'' protons in D_2O. On this basis an absolute configurational assignment of the H_5' and H_5'' protons was made. This assignment is the one assumed in the present study. However, since no absolute assignment has been attempted in DMSO the method of data treatment in this study is such that it is not dependent upon this assignment.

The H_2 and H_8 protons of the adenine base were assigned by comparison with selective deuteration studies, reported by Matsuura and Goto[29], Bullock and Jardetzky[30] and Schweizer et al[31], which have shown that the H_8 proton is found at lower field than H_2 in both D_2O and DMSO.

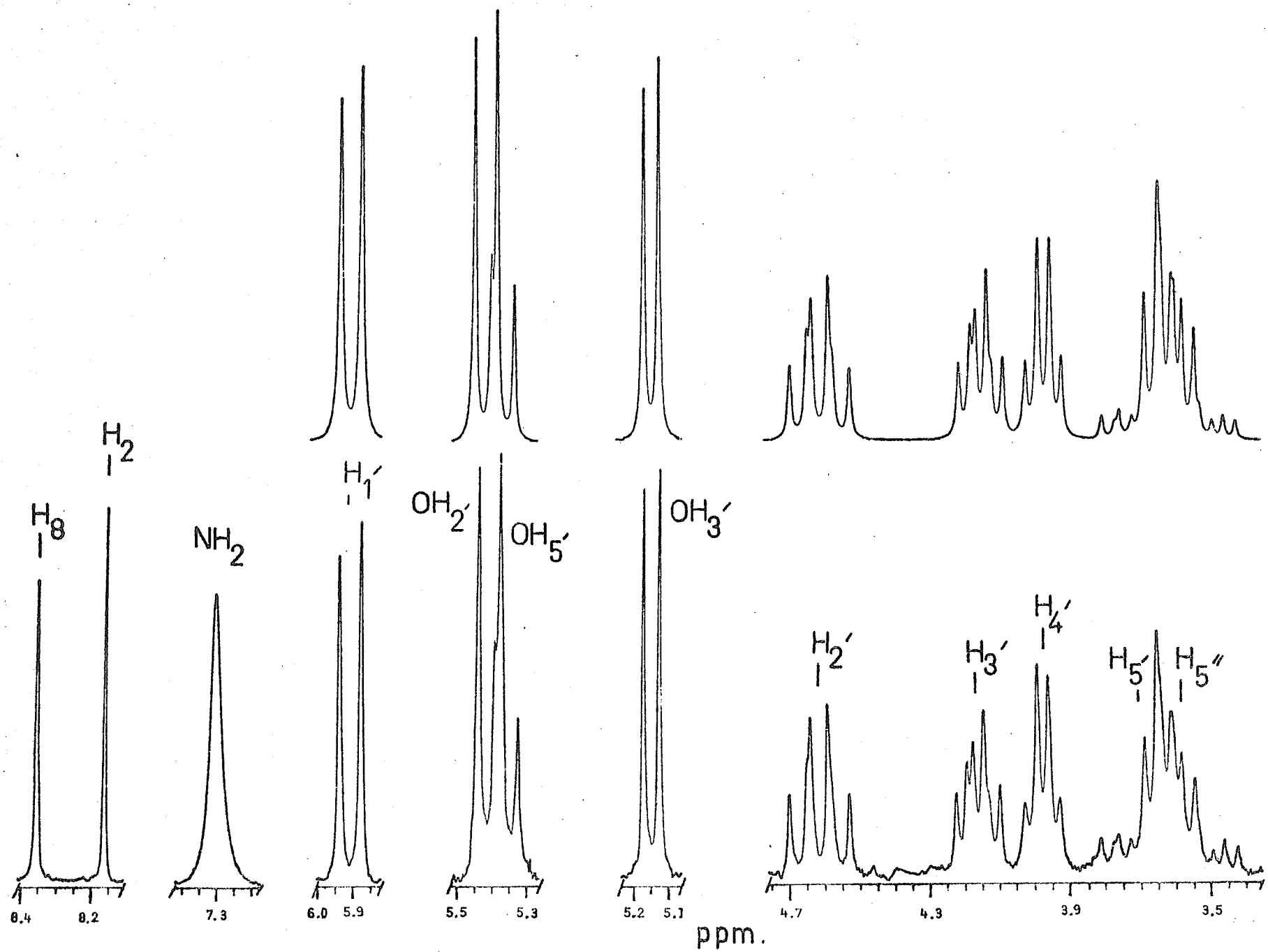
2. Exchangeable OH And NH Protons

The spectrum of adenosine and accompanying computer simulation in Figure 8 include the hydroxyl and amino resonances. The OH_2' and OH_3' protons give rise to doublets at 5.41 ppm and 5.15 ppm, respectively. The OH_5' proton resonance, centered at 5.38 ppm, results from unequal coupling to the methylene protons ($J_{5'05'}$ = 4.69 Hz. and $J_{5''05'}$ = 6.96 Hz.). Although the OH_2' and OH_5' resonances partially overlap in DMSO, the individual peaks were easily distinguishable when this portion of the spectrum was expanded.

The broad singlet at 7.30 ppm has been attributed to the amino protons of the base.

Figure 7

The 100 MHz. spectrum and computer simulation of adenosine in DMSO-d6, including hydroxyl and amino resonances.


H8
H2
NH2
H1′
OH2′
OH5′
OH3′
H2′
H3′
H4′
H5′
H5″
8.4
8.2
7.3
6.0
5.9
5.5
5.3
5.2
5.1
4.7
4.3
3.9
3.5
ppm.

B. 2'-Deoxyadenosine

1. Nonexchangeable Protons

The 2'-deoxyadenosine 100 MHz. spectrum and computer simulation are reproduced in Figure 8.

Batterham et al[32] have carried out a partial analysis of 2'-deoxyadenosine at 60 MHz. and reported values of $J_{1'2'}$ = 6.0 Hz. and $J_{1'2''}$ = 8.8 Hz. By substitution of these values into the Karplus equation[46] they have arrived at the dihedral angles required to yield these couplings. On this basis, Batterham et al have assigned an absolute configuration; $H_{2''}$ is on the same side of the furanose ring as the adenine base while $H_{2'}$ is on the opposite side. This assignment is the one assumed in the present study.

In 2'-deoxyadenosine the $H_{2'}$ and $H_{2''}$ protons are found substantially upfield at 2.73 ppm and 2.28 ppm, respectively, since the $C_{2'}$ atom is not bonded to oxygen. The $H_{2'}$ and $H_{2''}$ protons comprise the AB portion of an ABMX subspectrum (where M = $H_{1'}$ and X = $H_{3'}$).

2. Exchangeable OH And NH Protons

The undeuterated 2'-deoxyadenosine 100 MHz. spectrum is shown in Figure 9. The $OH_{3'}$ and $OH_{5'}$ resonances at 5.27 ppm and 5.20 ppm overlap. The small chemical shift differences between most hydroxyl protons in DMSO has been found to be a problem in the study of carbohydrates also[33]. It is interesting to note that the $H_{1'}$, $H_{2'}$, $H_{2''}$ and $H_{4'}$ proton resonances are virtually unchanged in both the

Figure 8

The 100 MHz. spectrum and computer simulation of 2'-deoxyadenosine in DMSO-d6, showing all nonexchangeable protons.

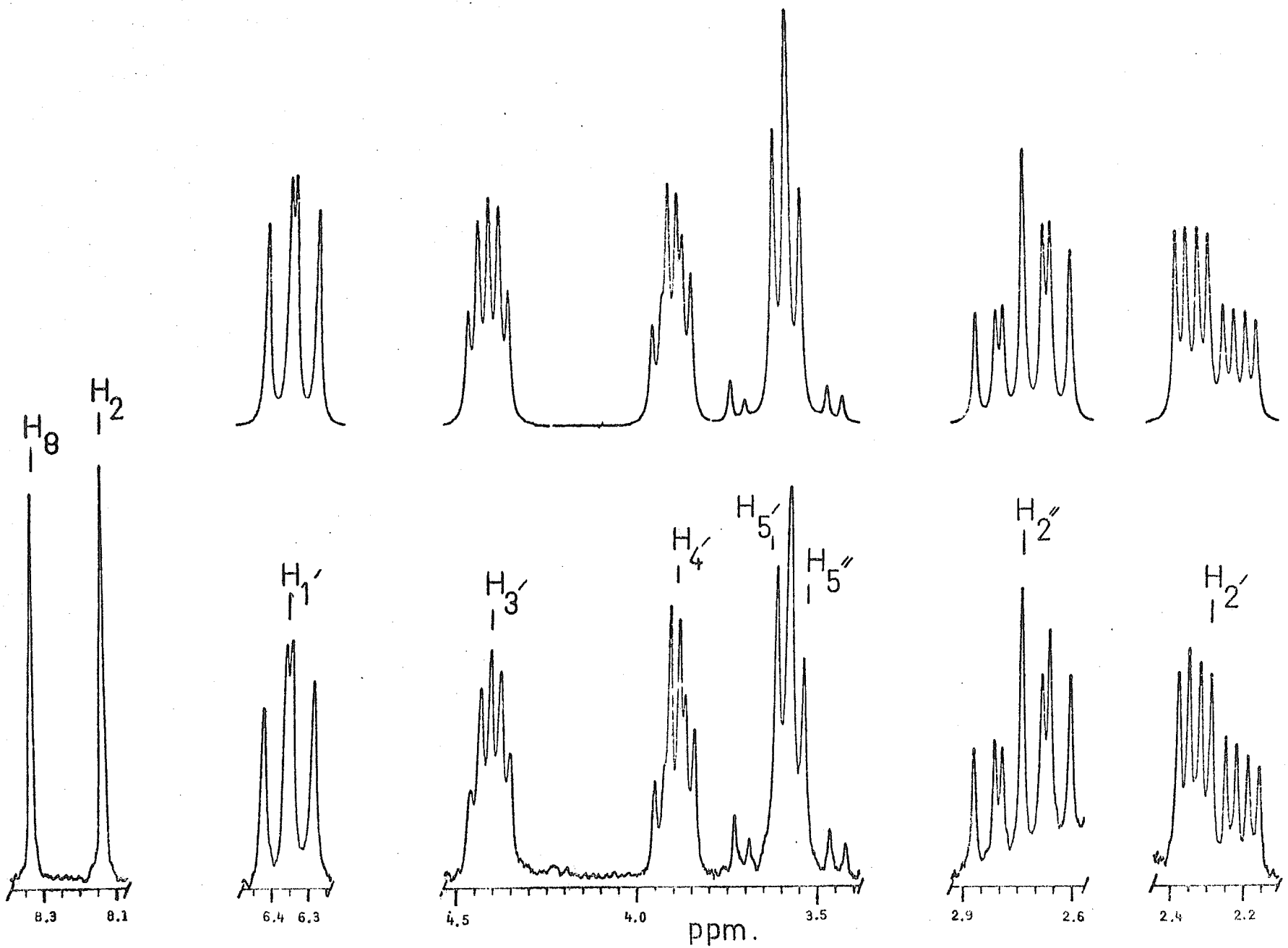
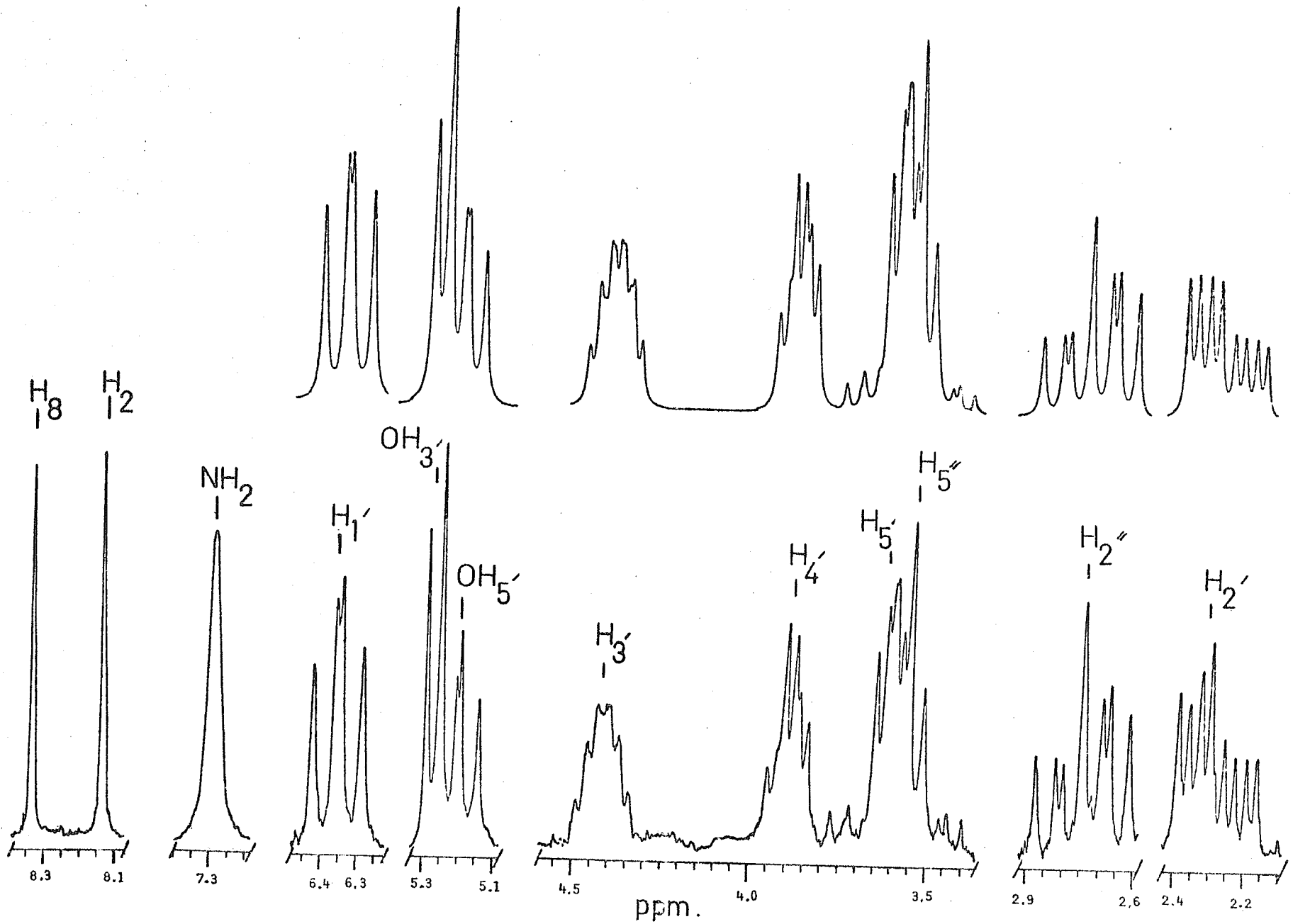

H8
H2
H1′
H3′
H4′
H5′
H5″
H2″
H2′
8.3
8.1
6.4
6.3
4.5
4.0
3.5
ppm.
2.9
2.6
2.4
2.2

Figure 9

The observed 100 MHz. spectrum and computer simulation of 2'-deoxyadenosine in DMSO-d6, including hydroxyl and amino resonances.


H8
H2
NH2
H1´
OH3´
OH5´
H3´
H4´
H5´
H5˝
H2˝
H2´
8.3
8.1
7.3
6.4
6.3
5.3
5.1
4.5
4.0
3.5
ppm.
2.9
2.6
2.4
2.2

deuterated and undeuterated spectra; this attests to the correctness of this assignment.

C. 8-Bromoadenosine

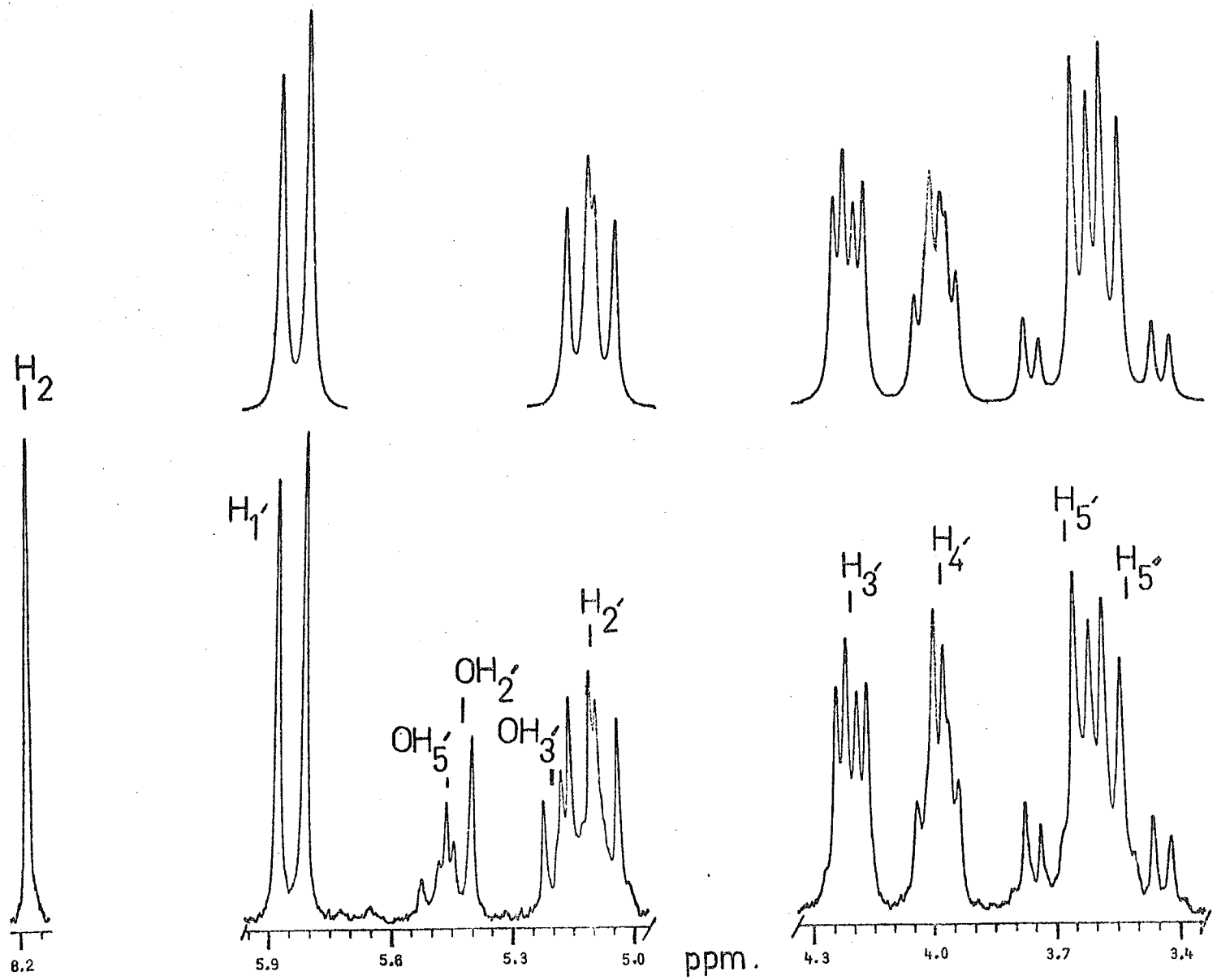
1. Nonexchangeable Protons

The furanose protons of this nucleoside also give well separated resonances at 100 MHz. (Figure 10). The chemical shifts of all the nonexchangeable protons of 8-bromoadenosine, except H_2', are within 0.04 ppm of their corresponding values in adenosine. The H_2' proton is shifted downfield by 0.47 ppm. The ABX pattern of the H_5' and H_5'' protons is better resolved for this compound due to a slight increase in the shift difference between H_5' and H_5''.

With the majority of nucleosides studied in DMSO it was found that the hydroxyl resonances were observed as sharp lines only when the sample and solvent were free of all water. 8-Bromoadenosine appears slightly anomalous in this respect. This nucleoside is relatively insoluble in D_2O and in order to exchange the hydroxyl protons for deuterons it was necessary to heat the sample for several hours in D_2O. This treatment failed to exchange all hydroxyl protons, as is evident in Figure 10, and a small amount of D_2O added to the sample failed to diminish the intensity of the hydroxyl 'impurity' peaks even after several hours. Many of the furanose resonances are broadened on either side due to coupling with the hydroxyl protons

Figure 10

The 100 MHz. spectrum and computer simulation of 8-bromoadenosine in DMSO-d6, showing all nonexchangeable protons.


H2
H1´
OH5´
OH2´
OH3´
H2´
H3´
H4´
H5´
H5´´
8.2
5.9
5.6
5.3
5.0
ppm.
4.3
4.0
3.7
3.4

which have not undergone exchange.

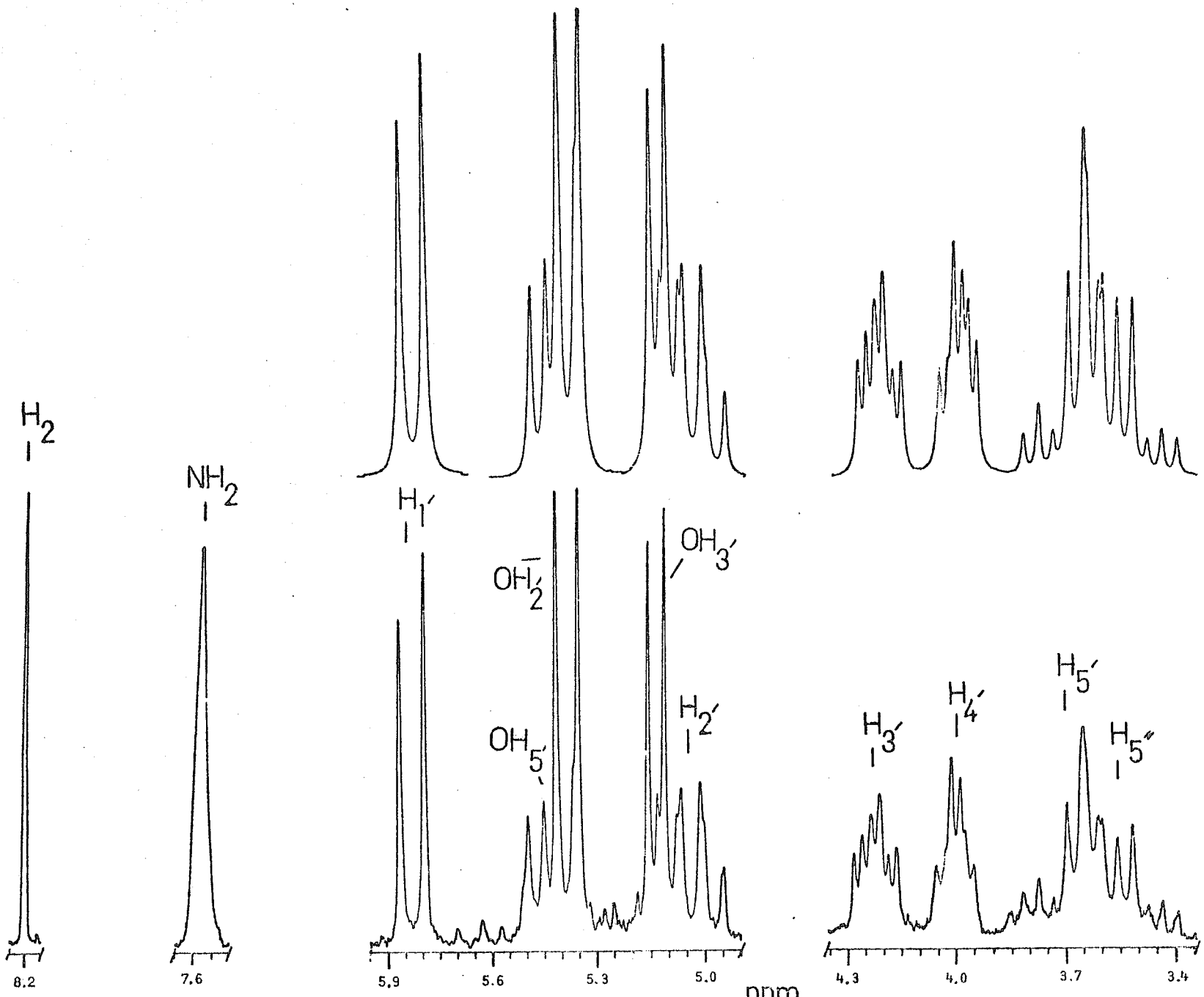
2. Exchangeable OH And NH Protons

The total 100 MHz. spectrum of 8-bromoadenosine in DMSO is presented in Figure 11. Due to the overlap of the OH_2' and OH_5' resonances, as well as broad line widths, the individual hydroxyl peaks were more readily distinguished in Figure 10.

Although the amino protons of adenosine and 2'-deoxy-adenosine are found within 0.03 ppm of each other, the amino protons of 8-bromoadenosine are shifted downfield 0.27 ppm with respect to adenosine.

Figure 11

The observed 100 MHz. spectrum and computer simulation of 8-bromoadenosine in DMSO-d6, including hydroxyl and amino resonances.


H2
NH2
H1′
OH2′
OH3′
OH5′
H2′
H3′
H4′
H5′
H5″
8.2
7.6
5.9
5.6
5.3
5.0
4.3
4.0
3.7
3.4
ppm

CHAPTER IV

Comparison Of Nucleoside Conformation In D_2O And DMSO

A. Sugar-Base Torsional Angle (ϕ_{CN})

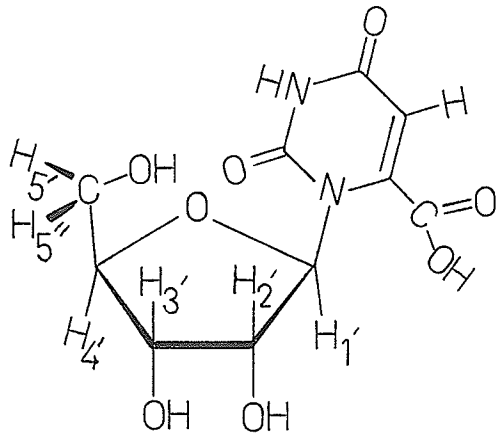
1. Pyrimidine Nucleosides

There is now substantial evidence that chemical shifts of certain ribose protons undergo specific changes when the pyrimidine nucleoside assumes the syn conformation in D_2O. Dugas et al[34] have studied β-cyanuric acid riboside (Figure 12) which must have a keto group over the furanose ring and may be considered as a model of the syn conformation. Compared to uridine, the H_2' and H_3' protons are deshielded 0.37 ppm and 0.16 ppm, respectively, while H_4', H_5' and H_5'' are shielded ~0.10 ppm. Schweizer et al[35] have combined ORD results and chemical shift data to show that 6-methyl pyrimidines exist in the syn conformation (Figure 12). These authors note an upfield shift of H_1' (0.25 ppm), a downfield shift of H_2' and H_3' (0.46 ppm and 0.16 ppm) and slight shielding of H_4', H_5' and H_5'' compared to uridine. Recently, X-ray studies have confirmed the syn conformation for the 6-methyl pyrimidines[36]. These characteristic shift differences in D_2O, between pyrimidines in the syn and anti conformations, are summarized in Table II.

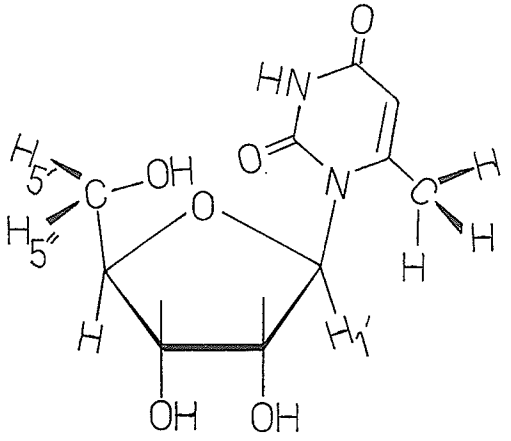
Hruska[37] has concluded that orotidine (6-carboxyuridine) is syn in D_2O on the basis of chemical shifts (Figure 12). The syn conformation for this compound is reasonable in view of the bulkiness of the carboxyl group. In Table III the chemical shifts of orotidine and uridine are compared in DMSO and D_2O. Note that the H_1' proton of orotidine is upfield, the H_2' and H_3' protons are downfield,

Figure 12

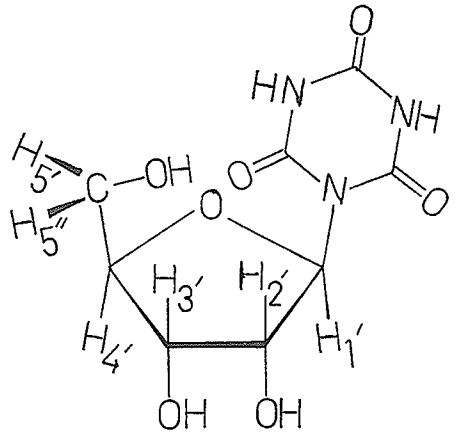
Structures of orotidine (syn), 6-methyluridine (syn) and β-cyanuric acid riboside.



OROTIDINE (Syn)



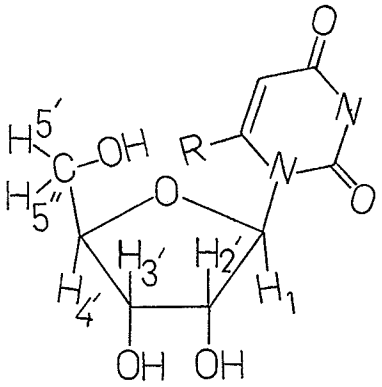
6-METHYLURIDINE (Syn)

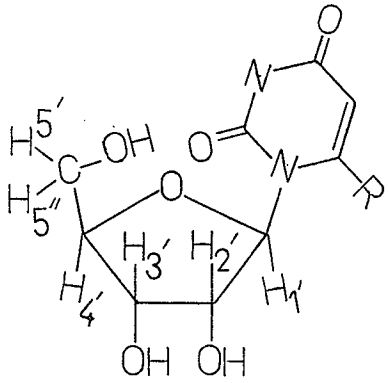


β-CYANURIC ACID RIBOSIDE

Table II

Shift Differences Between Pyrimidines in the Syn and Anti Conformations in D_2O.





Anti		Syn
ν_1'	upfield ~0.25 ppm →	ν_1'
ν_2'	downfield ~0.40 ppm →	ν_2'
ν_3'	downfield ~0.14 ppm →	ν_3'
ν_4'	upfield ~0.16 ppm →	ν_4'
ν_5'	upfield ~0.05 ppm →	ν_5'
ν_5''	upfield ~0.05 ppm →	ν_5''

Table III

Chemical Shifts of Uridine and Orotidine in D_2O and DMSO

	D_2O †			DMSO ††		
	Uridine (U)	Orotidine (O)	diff. ($\nu_U - \nu_O$)	Uridine (U)	Orotidine (O)	diff. ($\nu_U - \nu_O$)
ν_1' =	5.90	5.56	+0.34	5.78	5.60	+0.18
ν_2' =	4.34	4.74	-0.40	4.03	4.50	-0.47
ν_3' =	4.22	4.33	-0.11	3.97	4.09	-0.12
ν_4' =	4.13	3.94	+0.19	3.85	3.67	+0.18
ν_5' =	3.91	3.85	+0.06	3.62	3.61	+0.01
ν_5'' =	3.80	3.74	+0.06	3.55	3.44	+0.11

† chemical shifts in ppm downfield from internal DSS. (results in D_2O from ref. 37)

††chemical shifts in ppm downfield from internal TMS.

and the H_4', H_5' and H_5'' protons are slightly upfield from the corresponding uridine values in both solvents. The similarity in direction and magnitude of these shift changes suggests not only that uridine and orotidine are anti and syn, respectively, in DMSO, but also that the shift differences between the anti and syn conformations are independent of solvent. A reasonable explanation of the shift differences, and especially the large deshielding of the H_2' proton in the syn conformation, is based on the anisotropy† of the C_2 keto group[38]. There is a slight discrepancy in the magnitude of the shielding of the H_1' proton in DMSO and D_2O in the syn conformation (0.34 ppm in D_2O, 0.18 ppm in DMSO). However, the H_1' proton shift probably reflects any changes in the electronic configuration of the base and will also be most sensitive to changes in solvation about the carboxyl group of orotidine; therefore, it may not be unexpected that the H_1' proton shift fails to correlate exactly in DMSO and D_2O.

The majority of nucleosides reported have been examined in D_2O solution with shifts given relative to internal DSS (3-trimethylsilyl propane sulfonic acid, sodium salt). In the present study, DMSO-d6 was employed as the solvent and TMS as an internal standard. The result is that absolute values of chemical shifts cannot

† Due to the flexibility of nucleosides, it is difficult to establish which model of carbonyl anisotropy this evidence supports[51].

be compared between the two solvent systems. However, a comparison of relative shift differences between ribose protons in the two solvents should be meaningful. Similar approaches have been used by Glickson et al[39] and Schweizer[35] for the comparison of shifts obtained in different solvent systems.

In Table IV the chemical shifts of the ribose protons, (ν_i), relative to the H_2' proton (i.e. $\nu_2 - \nu_i$) are reported for various pyrimidine nucleosides in both D_2O and DMSO. The H_2' proton was chosen as the reference point since it is apparently less sensitive to solvation changes than H_1' (vide supra). Considering the characteristic shift changes discussed above, upon going from anti to syn, the shift differences for orotidine ($\nu_2 - \nu_i$) are used as a model for the syn conformation and those for uridine as a model for the anti conformation. The shift differences for most of the pyrimidines lie close to the values of uridine suggesting they are anti in DMSO. The shift differences for 6-methyluridine are similar to those of orotidine indicating this nucleoside is syn in DMSO. It is also interesting to note that the shift differences for any particular pyrimidine are very similar in both D_2O and DMSO. In summary, this chemical shift data certainly suggests that for pyrimidine nucleosides the change of solvent from D_2O to DMSO does not radically alter the sugar-base torsional angle.

shift diff. (ppm)	Uridine††		5-Iodo-uridine		5-Iodo-cytidine		5-COOEt-uridine		β-Pseudo-uridine	
	D_2O	DMSO	D_2O	DMSO	D_2O	DMSO	D_2O	DMSO	D_2O	DMSO
$\nu_2'-\nu_3'$	0.12	0.06	0.07	0.07	0.05	†	0.09	0.08	0.14	0.05
$\nu_2'-\nu_4'$	0.21	0.18	0.19	0.18	0.13	~.09	0.18	0.15	0.27	0.21
$\nu_2'-\nu_5'$	0.43	0.41	0.34	0.38	0.27	~.24	0.32	0.36	0.44	0.32
$\nu_2'-\nu_5''$	0.54	0.48	0.49	0.48	0.42	~.39	0.49	0.48	0.55	0.46

† the close proximity of ν_2' and ν_3' prevented an absolute assignment.

†† values in D_2O from :- ref. 44 (U), 44 (β-ψU), 37 (O), (5IU, 5IC, and 5COOEtU from Hruska et al, unpublished)

shift diff. (ppm)	Orotidine		6-Methyl-uridine†	
	D_2O	DMSO	D_2O	DMSO
$\nu_2'-\nu_3'$	0.41	0.41	0.42	0.49
$\nu_2'-\nu_4'$	0.80	0.83	0.81	0.84
$\nu_2'-\nu_5'$	0.88	0.88	~0.97	~1.03
$\nu_2'-\nu_5''$	0.99	1.05	~0.97	~1.03

† values from ref. 58.

Table IV

Relative Shift Differences Between the Ribose Protons in D_2O and DMSO

Further support in the case of β-psuedouridine, for the absence of a significant change in ϕ_{CN}, is offered in the presence of a long-range coupling constant. Hruska et al[40] have reported an allylic coupling between the H_1' proton of the furanose and the H_6 proton of the base, $J_{1'6} = 0.8$ Hz. In DMSO this coupling is 0.7 Hz. Allylic coupling constants are related to the dihedral angle between the relevant HCC'H' planes and can vary between ±3 Hz.[41] The apparent similar value of this coupling in D_2O and DMSO also supports the idea that very little change of ϕ_{CN} accompanies this change of solvent.

2. Purine Nucleosides

Information of the sugar-base torsional angle of purine nucleosides has been difficult to obtain in solution. Danyluk and Hruska[42] have demonstrated that the ionization of the phosphate of 5'-adenosine monophosphate results in a deshielding of the H_8 proton but does not affect the H_2 proton; this requires that the nucleotide be in the anti conformation. ORD-CD results in D_2O have indicated that a change of ϕ_{CN} takes place when the purine bears a bulky substituent at the C_8 position[13]. X-Ray studies have shown that 8-bromoadenosine is syn in the solid state[43]. In DMSO, the chemical shifts of the ribose protons in 8-bromoadenosine are within 0.05 ppm of their corresponding values in adenosine, except for H_2' (see Table I). The H_2' proton of 8-bromoadenosine is deshielded 0.48 ppm. A study of molecular models demonstrates that

the H_2' proton and the bromine atom may be in close proximity in both the *syn* and *anti* conformations. It is also possible that the deshielding does not arise from a through space effect of the bromine but simply arises from some specific change in ϕ_{CN}. However, if the bromine atom does cause rotation of the base, the ribose proton chemical shifts do not reflect this rotation, except possibly H_2'.

B. The Exocyclic CH_2OH Group

The three classical staggered conformations about the C_4'-C_5' bond are shown in Figure 3. Although these rotamers probably do not represent true energy minima due to the neglect of the oxygen-oxygen repulsions, the conformational difference between the classical rotamers and the minimum energy rotamers is not expected to be large[44]. In solution rotation about the C_4'-C_5' bond will be rapid, however, the time spent in the staggered conformations is probably long compared to the time passing through the eclipsed intermediates. Therefore, the observed coupling constants, $J_{4'5'}$ and $J_{4'5''}$, will be weighted time-averages of the preferred conformations.

An absolute configurational assignment of the H_5' and H_5'' protons has not been assumed in DMSO. Therefore, the *gauche-trans* conformation will not be distinguished from the *trans-gauche*. For this reason it is more convenient to consider only the sum $J_{4'5'} + J_{4'5''}$; the smallest value for this sum should be observed for the *gauche-gauche* conformation and any increase will indicate that the hydroxyl group is swinging away from the furanose (i.e. *gt* or *tg*). Hruska

et al[45] have used the following equation to calculate the fraction of the rotamer population in the gg conformation:

$$(1) \qquad P_{gg} = \frac{12 - \Sigma}{8} \qquad (\Sigma = J_{4'5'} + J_{4'5''})$$

The values of P_{gg}, calculated from equation 1, are presented in Table V for several nucleosides in DMSO and D_2O. The effect of DMSO on all pyrimidine nucleosides listed is a slight increase in preference for the gg conformation, while the purine nucleosides, adenosine and 2'-deoxyadenosine, show a slight decrease in preference for the gg conformation. However, in general the effects are not large and those nucleosides which favor the gg conformation in D_2O also favor this conformation in DMSO.

C. The Furanose Conformation

The most successful approach to the determination of the furanose conformation in solution has been through the use of the Karplus equation[46]. This equation allows the ribose vicinal coupling constants to be related to the relevant HCC'H' fragments.

$$(2) \qquad J(\theta) = \begin{cases} J^0 \cos^2\theta - C & \text{for } 0° < \theta < 90° \\ J^{180} \cos^2\theta - C & \text{for } 90° < \theta < 180° \end{cases}$$

(where J^0, J^{180} and C are constants)

However, due to the sensitivity of the vicinal coupling constants to substituents, bond distortion, etc., the Karplus equation is most useful when similar systems are being compared.

TABLE V

Nucleoside	P_{gg}(DMSO)	P_{gg}(D_2O)	P_{gg}(DMSO-D_2O)
a. Pyrimidines :			
Uridine	0.68	0.58	+0.10
5-Iodouridine	0.86	0.73	+0.13
5-Iodocytidine	0.88	0.84	+0.04
5-Ethylcarboxy-uridine	0.88	0.84	+0.04
2'-Deoxyuridine	0.59	0.44	+0.15
2'-Deoxycytidine	0.56	0.41	+0.15
β-Psuedouridine	0.68	0.53	+0.15
b. Purines :			
Adenosine	0.60	0.74	-0.14
2'-Deoxyadenosine	0.46	0.59	-0.13

Conformation Populations About the C_4'-C_5' Bond in D_2O and DMSO.

Smith and Jardetzky[47] have estimated the dihedral angles required for the 20 possible endo and exo conformations and from these angles have calculated the expected coupling constants. Hruska et al[40,48] have reported the complete analyses of several ribonucleosides in D_2O and concluded that the observed ribose coupling constants, $J_{1'2'}$, $J_{2'3'}$ and $J_{3'4'}$ are best explained by assuming a C_2'-endo, C_3'-exo $\leftrightarrow$ C_3'-endo, C_2'-exo type of conversion. In solution it is doubtful that a significant barrier separates the C_2'-endo conformation from the C_3'-exo, or the C_3'-endo conformation from the C_2'-exo (Figure 13). However, a change from the C_2'-endo to the C_3'-endo involves the eclipsing of adjacent hydroxyl groups, therefore, a time-averaged preference for either the C_2'-endo (C_3'-exo) or the C_3'-endo (C_2'-exo) conformation may be expected. Hall[49] has suggested that the barrier to conformational inversion of the furanose ring may be of a magnitude similar to that for the inversion of cyclopentane, ie. 3-4 kcal/mole. This means that the inversion is fast on the NMR time scale and it is only meaningful to discuss time-averaged conformations.

To monitor any change in preference for the C_2'-endo or C_3'-endo conformations, Hruska et al[50] have chosen $J_{1'2'}$ and $J_{3'4'}$. Any change in conformation should result in one of these couplings increasing and the other decreasing, as a study of molecular models will indicate. The ribose coupling constants are listed in Table VI for several

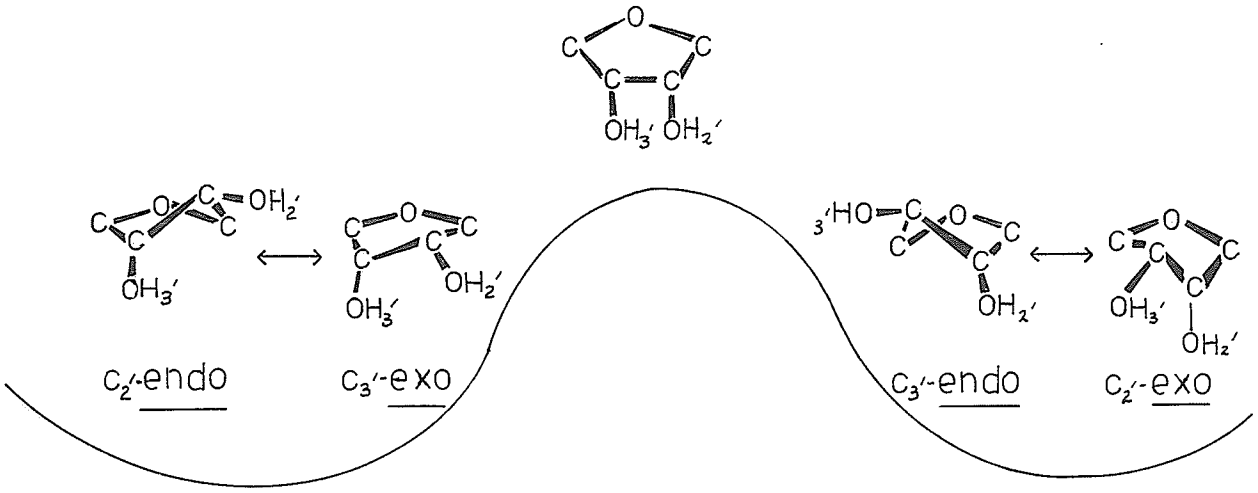


Figure 13

Interconversion of the C_2'-endo, C_3'-exo ↔ C_3'-endo, C_2'-exo conformations of the ribose.

Table VI

Comparison of the Ribose Coupling Constants in D_2O and DMSO

Group A :-

	Uridine			5-Iodo-uridine		
	D_2O	DMSO	diff.	D_2O	DMSO	diff.
$J_{1'2'}$=	4.40	5.50	-1.10	3.41	4.60	-1.19
$J_{2'3'}$=	5.30	5.23	+0.07	5.27	5.10	+0.17
$J_{3'4'}$=	5.50	3.57	+1.93	6.30	4.40	+1.90

	5-Iodo-cytidine			5-COOEt-uridine		
	D_2O	DMSO	diff.	D_2O	DMSO	diff.
$J_{1'2'}$=	2.70	3.50	-0.80	2.38	4.02	-1.64
$J_{2'3'}$=	4.91	5.10	-0.19	4.90	4.74	+0.16
$J_{3'4'}$=	6.97	5.30	+1.67	7.25	5.14	+2.11

Group B :-

	Adenosine			β-Pseudo-uridine			Orotidine		
	D_2O	DMSO	diff.	D_2O	DMSO	diff.	D_2O	DMSO	diff.
$J_{1'2'}$=	5.93	6.12	-0.19	5.00	4.40	+0.60	3.60	3.60	0.00
$J_{2'3'}$=	5.25	4.87	+0.38	5.00	4.95	0.05	6.30	6.05	+0.25
$J_{3'4'}$=	3.14	3.22	-0.08	5.20	5.64	-0.44	7.00	5.95	+1.05

nucleosides in DMSO and compared with the corresponding values in D_2O. The nucleosides in Group A of this table show characteristic changes in $J_{1'2'}$ and $J_{3'4'}$ upon changing solvents; in DMSO $J_{1'2'}$ decreases by an average of 1.18 Hz., $J_{3'4'}$ increases by an average of 1.90 Hz. and $J_{2'3'}$ is relatively unaffected. These results indicate an increased preference for the C_2'-endo (C_3'-exo) conformation in DMSO. The nucleosides in Group B do not show similar furanose conformational changes; undoubtedly they are not adequately described by this rather naive model.

Recently attention has been drawn to a correlation between the furanose conformation and rotation about the C_4'-C_5' bond, in aqueous solution[50]; the gauche-gauche conformation is favored if the ribose, or deoxyribose, is in the C_3'-endo conformation and less favored if the sugar is puckered C_2'-endo (Figure 14). It has been difficult to explain this correlation because the nucleosides displaced along the line, farthest from the position of uridine, are the same nucleosides that tend to self-associate in aqueous solution, ie. the halogenated pyrimidines[22,23].

As shown in Figure 14, the correlation of furanose conformation with exocyclic bond rotation also exists in DMSO. The general effect of the DMSO has been to shift the correlation in the direction of the C_2'-endo conformation. The halogenated pyrimidines still show the strongest preference for the C_3'-endo gauche-gauche conformation, which certainly suggests that the correlation is solvent

Figure 14a

Plot of $J_{4'5'} + J_{4'5''}$ vs. $J_{3'4'}$ for a series of nucleosides in D_2O and DMSO-d6. The results in D_2O were obtained from ref. 50 & 52.

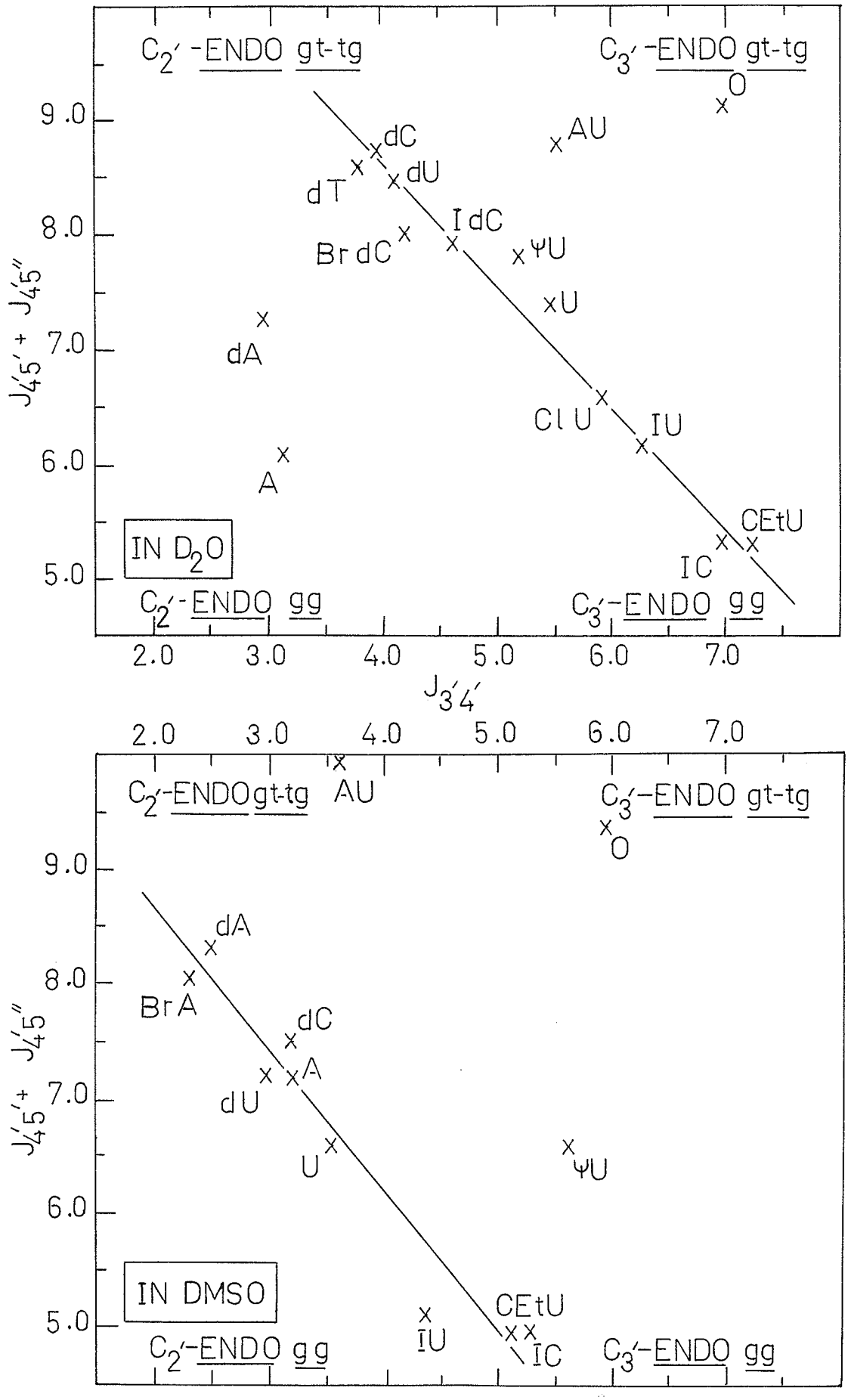
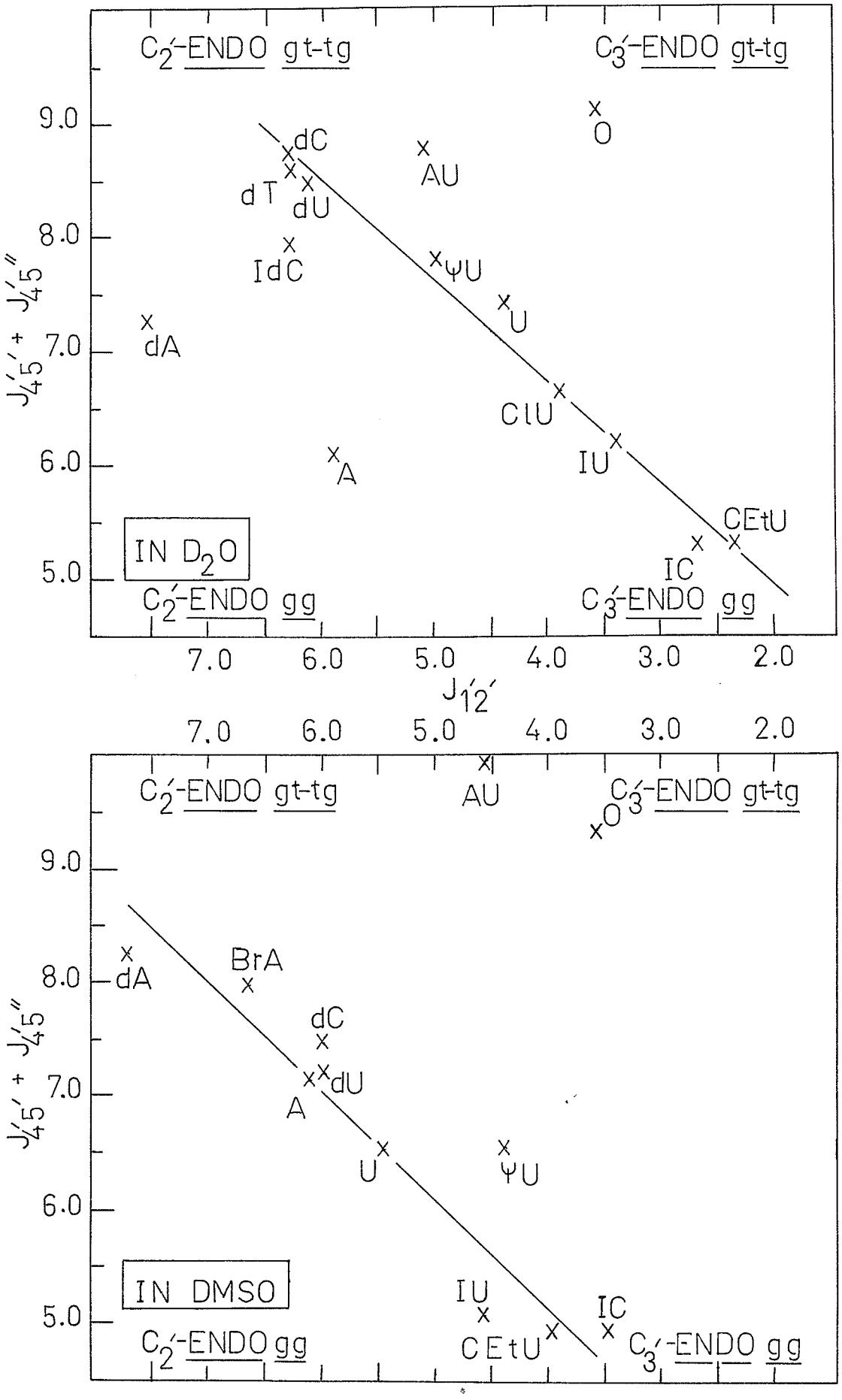

C2′-ENDO gt-tg
C3′-ENDO gt-tg
O
AU
dC
dU
dT
IdC
BrdC
ΨU
xU
dA
ClU
IU
A
CEtU
IC
IN D2O
C2′-ENDO gg
C3′-ENDO gg
J4′5′ + J4′5″
J3′4′
2.0
3.0
4.0
5.0
6.0
7.0
9.0
8.0
7.0
6.0
5.0
C2′-ENDO gt-tg
AU
C3′-ENDO gt-tg
O
dA
BrA
dC
A
dU
U
ΨU
IN DMSO
CEtU
IU
IC
C2′-ENDO gg
C3′-ENDO gg

Figure 14b

Plot of $J_{4'5'} + J_{4'5''}$ vs. $J_{1'2'}$ for a series of nucleosides in D_2O and DMSO-d6. The results in D_2O were obtained from ref. 50 & 52.


C2′-ENDO gt-tg
C3′-ENDO gt-tg
dC
dT
dU
AU
O
IdC
ΨU
U
dA
ClU
IU
A
CEtU
IC
IN D2O
C2′-ENDO gg
C3′-ENDO gg
9.0
8.0
7.0
6.0
5.0
J′45′ + J′45″
7.0
6.0
5.0
4.0
3.0
2.0
J1′2′
BrA
dA
dC
dU
A
U
ΨU
IU
IC
CEtU
IN DMSO

independent and, therefore, not the result of molecular association. Apparently the conformation of the sugar is altered through some intramolecular effect of the C_5-substituents.

The purine nucleosides, adenosine and 2'-deoxyadenosine, correlate in DMSO while they do not in D_2O. This possibly indicates that the ribose conformation of the purines in D_2O is influenced by self-association.

CHAPTER V

Hydroxyl Group Orientation

Dimethyl sulphoxide is able to form strong hydrogen bonds with hydroxyl and amino protons. However, unlike water, DMSO has no exchangeable protons and can participate only as a proton acceptor. Therefore, the exchange process is substatially slowed in dimethyl sulphoxide and the exchangeable proton resonances are often well resolved.

Chapman and King[54] were among the first to report chemical shifts and coupling constants for hydroxyl protons. Fraser et al[55] have rigorously demonstrated that a Karplus relationship does hold for J_{HCOH}. Values of J^{trans}_{HCOH} and J^{cis}_{HCOH} have been difficult to obtain because of the problem of restricting rotation about the C-O bond. Bauld and Rim[56] were able to establish a value for J^{trans}_{HCOH} of 12-13 Hz. in carbon tetrachloride for an alcohol whose hydroxyl proton is strongly intramolecularly hydrogen bonded. The average value of J_{HCOH} for various alcohols is 4.5-5.5 Hz.; this range has been assumed to be that due to 'free-rotation' about the C-O bond.

The chemical shifts and coupling constants of the ribose hydroxyl protons are listed in Table 1. In the ribonucleosides for which the hydroxyl protons could be unequivocally assigned, the average value of $J_{2'0_{2'}}$ is 5.87 Hz. while the average value of $J_{3'0_{3'}}$ is 4.67 Hz. Moniz et al[57] have reported that J_{HCOH} increases as the electronegativity of the β substituents increases, therefore, the larger value of $J_{2'0_{2'}}$ may arise due to the presence of the pyrimidine on purine base β to the $C_{2'}$

Leaf blank to correct numbering.

hydroxyl proton. The slight deshielding of the C_2' hydroxyl proton (with respect to the C_3' hydroxyl proton) also may result from the electronegativity effects of the base, or may indeed reflect slight differences in the hydrogen bonding of the two protons.

Rotation about the C-O bond is probably fast on the NMR time-scale, therefore, the most useful method of interpreting the J_{HCOH} values is in terms of weighted time-averages of the classical conformations (Figure 15). Rearrangement of the population equation used by Danyluk and Davies[28] gives the following:

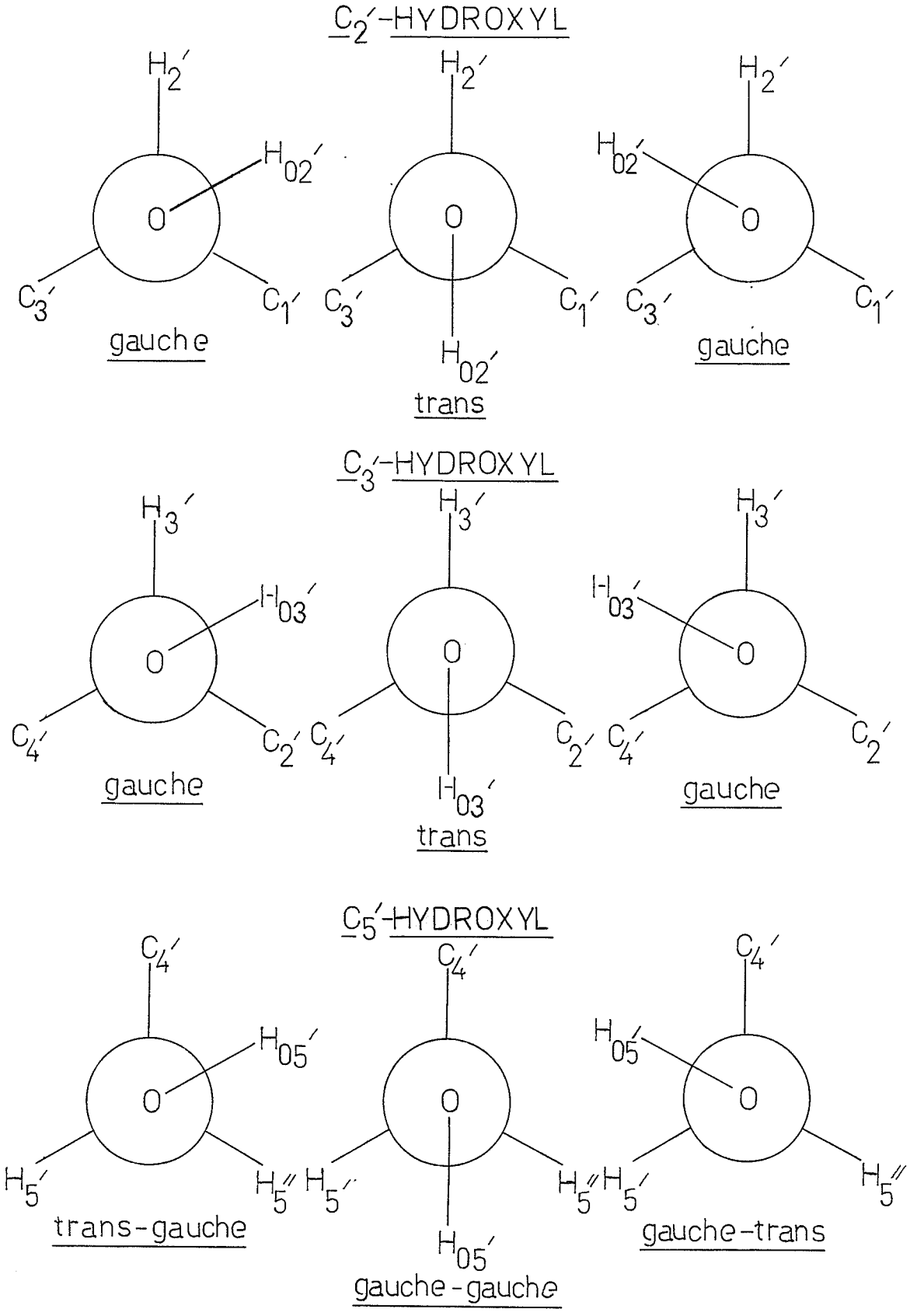
$$(3) \qquad P_{\underline{gauche}} = \frac{12.1 - J_{HCOH}}{10.0}$$

If substituent effects are small, the couplings indicate that the C_2' and C_3' hydroxyl protons spend ~64% and ~75% of their time in the gauche conformation.

Danyluk and Davies[28] observed only triplets (or pseudotriplets) for the C_5' hydroxyl proton in benzene/DMSO mixtures and concluded that there is free rotation about the C_5'-O_5' bond. In the present study $J_{5'05'}$ and $J_{5''05'}$ were found to be approximately equal for any particular pyrimidine nucleoside, however, these couplings differed by as much as 4.7 Hz. for the purines. Therefore, it may be concluded that the C_5' hydroxyl proton spends much more time in the gauche-trans and/or the trans- gauche conformations than do the C_5' hydroxyl protons of the pyrimidine nucleosides (see Figure 15).

Figure 15

The classical staggered conformations of the sugar hydroxyl groups.


C2′-HYDROXYL
H2′
H02′
O
C3′
C1′
gauche
H2′
O
C3′
C1′
H02′
trans
H2′
H02′
O
C3′
C1′
gauche
C3′-HYDROXYL
H3′
H03′
O
C4′
C2′
gauche
H3′
O
C4′
C2′
H03′
trans
H3′
H03′
O
C4′
C2′
gauche
C5′-HYDROXYL
C4′
H05′
O
H5′
H5″
trans-gauche
C4′
O
H5′
H5″
H05′
gauche-gauche
C4′
H05′
O
H5′
H5″
gauche-trans

CHAPTER VI

Summary and Conclusions

The proton magnetic resonance spectra of several nucleosides in dimethyl sulphoxide have been analyzed at ambient temperature. The chemical shifts and coupling constants are interpreted in terms of various conformational parameters common to most nucleosides. The conformations are compared in D_2O and DMSO as an initial step in the determination of the extent of solvent effects upon nucleoside conformation.

The sugar-base torsional angle (ϕ_{CN}) of pyrimidines is not radically altered by the change from D_2O to DMSO; orotidine and 6-methyl uridine are <u>syn</u> in D_2O and DMSO while uridine, β-pseudo uridine, and the C_5 substituted pyrimidines are <u>anti</u> in both solvents. Apparently a bulky substituent at the C_6 position of the base destabilizes the <u>anti</u> conformation whereas a bulky C_5 group (eg. -COOEt) has little effect. The ribose proton chemical shifts reflect ϕ_{CN} in both solvents; the shift differences between the <u>syn</u> and <u>anti</u> conformation probably arise from the anisotropy of the C_2 keto group.

The furanose is described in terms of the rotation about the C_4'-C_5' bond and the ring puckering. The <u>gauche-gauche</u> conformer is favored by most nucleosides studied in DMSO and D_2O, although there are slight differences in the conformer populations in the two solvents. The ribose coupling constants of the pyrimidines indicate that the puckering of the furanose is shifted toward the C_2'-<u>endo</u> (C_3'-<u>exo</u>) conformation in dimethyl sulphoxide. The ribose

puckering and the rotation about the C_4'-C_5' bond correlate in both D_2O and DMSO suggesting that the reason for this correlation is intra- rather than intermolecular.

The hydroxyl proton chemical shifts and coupling constants (J_{HCOH}) are reported for many of the nucleosides studied. The OH_2' and OH_3' proton couplings are in the range given by 'freely-rotating' alcohols. Although the OH_5' proton of pyrimidines couples by approximately the same amount to both C_5' methylene protons, the OH_5' proton of purines couples differently to each of the methylene protons. This evidence suggests that free-rotation of the OH_5' group is hindered in the case of purine nucleosides in DMSO, perhaps as a result of hydrogen bonding with the base.

Bibliography

1. Cunningham,K.G., Hutchinson,S.A., Manson,W., and Spring,F.S., J.Chem.Soc. 2299 (1951).

2. Donahue,J., and Trueblood,K.N., J.Mol.Biol. 2, 263 (1960).

3. Sundaralingam,M., Biopolymers 7, 821 (1969).

4. Blackburn,B.J., Grey,A.A., Smith,I.C.P., and Hruska,F.E., Can.J.Chem. 48, 2866 (1970).

5. Sundaralingam,M., Biopolymers 7, 821 (1969) (and references therein).

6. Kapuler,A.M., Monny,C., and Michelson,A.M., Biochim. Biophys. Acta. 217, 18 (1970).

7. Reeke,G.N.Jr., and Marsh,R.E., Acta.Cryst. 20, 703 (1966).

8. Rohrer,D., and Sundaralingam,M., Chem.Commun. 746 (1968).

9. Saenger,W., and Scheit,K.H., J.Mol.Biol. 50, 153 (1970).

10. Haschemeyer,A.E.V., and Sobell,H.M., Acta.Cryst. 19, 125 (1965).

11. Rogers,G.T., and Ulbricht,F.L.V., Biochem.Biophys. Res.Comm. 39, 414 (1970).

12. Miles,D.W., Robins,M.J., Robins,R.K., Winkley,M.W., and Eyring,H., J.Amer.Chem.Soc. 91, 824 (1969).

13. Ikehara,M., Uesugi,S., and Yoshida,K., Biochemistry 11, 830 (1972).

14. Miles,D.W., Robins,M.J., Robins,R.K., Winkley,M.W., and Eyring,H., J.Amer.Chem.Soc. 91, 831 (1969).

15. Miles,D.W., Townsend,L.B., Robins,M.J., Robins,R.K., Inskeep,W.H., and Eyring,H., J.Amer.Chem.Soc. 93, 1600 (1971).

16. Hruska,F.E., Ogilvie,K.K., Smith,A.A., and Wayborn,H., Can.J.Chem. 49, 2449 (1971).

17. Hruska,F.E., Grey,A.A., and Smith,I.C.P., J.Amer.Chem. Soc. 92, 4088 (1970).

18. Schweizer,M.P., Witkowski,J.T., and Robins,R.K. J.Amer.Chem.Soc. 93, 277 (1971).

20. Danyluk,S.S., and Hruska,F.E., Biochemistry 7, 1038 (1968).

21. Iball,J., Morgan,C.H., and Wilson,H.R., Proc.Roy. Soc. A.302, 225 (1968).

22. Ts'o,P.O.P., Fine Structure of Proteins and Nucleic Acids, Marcel Dekker, Inc., New York, 1970, Part 2, p.49.

23. Broom,A.D., Schweizer,M.P., Ts'o,P.O.P., J.Amer. Chem.Soc. 89, 3612 (1967).

24. Chapman,O.L., and King,R.W., J.Amer.Chem.Soc. 86, 1256 (1964).

25. Casu,B., Reggiani,M., Gallo,G.G., and Vigevani,A., Tetrahedron 22, 3061 (1966).

26. Bauld,N.L., and Rim,Y.S., J.Org.Chem. 33, 1303 (1968).

27. Remin,M., and Shugar,D., Biochem.Biophys.Res.Comm. 48, 636 (1972).

28. Davies,D.B., and Danyluk,S.S., Can.J.Chem. 48, 3112 (1970).

29. Matsuura,S., and Goto,T., Tetrahedron Letters 22, 1499 (1963).

30. Bullock,F.J., and Jardetzky,O., J.Org.Chem. 29, 1988 (1964).

31. Schweizer,M.P., Chan,S.I., Helmkamp,G.K., and Ts'o,P.O.P., J.Amer.Chem.Soc. 86, 696 (1964).

32. Blakley,R.L., Ghambeer,R.K., Batterham,T.J., and Brownson,C., Biochem.Biophys. Res.Comm. 24, 418 (1966).

33. Casu,B., Reggiani,M., Gallo,G.G., and Vigevani,A., Tetrahedron 22, 3061 (1966).

34. Dugas,H., Blackburn,B.J., Robins,R.K., Deslauriers,R., and Smith,I.C.P., J.Amer.Chem.Soc. 93, (1971).

35. Schweizer,M.P., Witkowski,J.T., and Robins,R.K., J.Amer.Chem.Soc. 93, 277 (1971).

36. Suck,D., and Saenger,W., J.Amer.Chem.Soc. 94, 6520 (1972).

37. Hruska, F.E., J.Amer.Chem.Soc. 93, 1795 (1971).

38. Schweizer,M.P., Witkowski,J.T., and Robins,R.K., J.Amer.Chem.Soc. 93, 277 (1971).

39. Glickson,J.D., Urry,D.W., and Walter,R., Proc.Nat. Acad.Sci. USA 69, 2566 (1972).

40. Hruska,F.E., Grey,A.A., and Smith,I.C.P., J.Amer. Chem.Soc. 92, 4088 (1970).

41. Sternhill,S., Quart.Rev.(London) 23, 236 (1969).

42. Danyluk,S.S., and Hruska,F.E., Biochemistry 7, 1038 (1968).

43. Rogers,G.T., and Ulbricht,F.L.V., Biochem.Biophys. Res.Comm. 39, 414 (1970).

44. Blackburn,B.J., Grey,A.A., Smith,I.C.P., and Hruska,F.E., Can.J.Chem. 48, 2866 (1970).

45. Hruska,F.E., Wood,D.J., and Dalton,J., (unpublished results).

46. Karplus,M., J.Chem.Phys. 30, 11 (1959).

47. Smith,M., and Jardetzky,C.D., J.Mol.Spectrosc. 28, 70 (1968).

48. Blackburn,B.J., Grey,A.A., Smith,I.C.P., and Hruska,F.E., Can.J.Chem. 48, 2866 (1970).

49. Hall,L.D., Steiner,P.R., and Pedersen,C., Can.J.Chem. 48, 1155 (1970).

50. Hruska,F.E., Smith,A.A., Dalton,J.G., J.Amer.Chem. Soc. 93, 4334 (1971).

51. Karabatsos,G.J., Sonnichsen,G.C., Hsi,N., Fenoglio,D.J., J.Amer.Chem.Soc. 89, 5067 (1967).

52. Hruska,F.E., Proceedings, International Symposium on the Conformation of Biological Molecules and Polymers, Symposia on Quantum Chemistry and Biochemistry, Eds. B. Pullman and E.D.Bergman, Vol. V, Academic Press (in press) 1972.

53. Haigh,C.W., and Williams,J.M., J.Mol.Spectrosc. 32, 398 (1969).

54. Chapman,O.L., and King,R.W., J.Amer.Chem.Soc. 86, 1256 (1964).

55. Fraser,R.R., Kaufman,M., Morand,P., and Govil,G., Can.J.Chem. 47, 403 (1969).

56. Bauld,N.L., and Rim,Y.S., J.Org.Chem. 33, 1303 (1968).

57. Moniz,W.B., Poranski,C.F.,Jr., and Hall, T.N., J.Amer.Chem.Soc. 88, 190 (1966).

58. Schweizer,M.P., Banta,E.B., Witkowski,J.T., and Robins,R.K., J.Amer.Chem.Soc. 95, (in press)(1973).