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CONFORMATIONAL STUDIES ON THE 2′-DEOXY NUCLEOSIDES

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ABSTRACT

Variable-temperature 220-MHz n m r studies conducted on the 2′-deoxy derivatives of cytidine, thymidine, uridine, adenosine, guanosine, inosine and, to a limited extent, their 5′-phosphate disodium salts, allowed accurate proton-shift and coupling data to be obtained for the 2-deoxy-*β*-D-*erythro*-pentofuranosyl portion of the molecule Conformational analysis, aided by the DAERM method, indicated that the sugar moiety of these molecules has a favored conformation of ${}^{2}V \rightleftarrows {}^{2}T_{3} \rightleftarrows V_{3}$ and an alternative favored conformation of ${}^{0}V \rightleftarrows {}^{0}T_{4} \rightleftarrows V_{4}$

INTRODUCTION

Relatively few n m r studies of 2′-deoxy nucleosides have been undertaken (see ref 1 and references therein), in spite of the importance of this class of compounds. Our interest in the deoxyfuranose system[2] and the availability of 220-MHz facilities has led us to investigate these molecules and elucidate the conformational properties that the 2′-deoxy-*β*-D-*erythro*-pentofuranosyl moiety exhibits The potential flexibility of the furanoid ring, and the difficulty in analyzing highly coupled, second-order spectra having seven intercoupled spins, made this not an easy problem With the 220-MHz facility, analysis of the spectra was greatly simplified Application of the "Dihedral Angle Estimation by the Ratio Method" (DAERM)[3] enabled an evaluation of the time-averaged angles subtended by H-1′ and H-3′, which were coupled into the C-2′(deoxy) methylene group From these angles, and their variation with temperature over a range of 80°, a description of the conformational properties of the sugar ring has been developed

RESULTS AND DISCUSSION

LAOCN III[4] analysis of the 220-MHz n m r spectra yielded the chemical shifts (Table I) and coupling constants (Table II) of the protons in the sugar moiety of the deoxy nucleosides studied (Figs 1 and 2)

The spectra measured at 80° were well resolved, even to the extent that a long-range coupling from H-1′ to H-3′ of about 0 5 Hz was often observable. At lower

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TABLE I

CHEMICAL SHIFTS[a]

Compound	*Solvent*	*Temp (deg)*	*H-1'*	*H-2'a*	*H-2'b*	*H-3'*	*H-4'*	*H-5'a*	*H-5'b*
Purine derivatives									
Adenosine (1)	C_5D_5N	80	3 20	6 88	7 31	4 94	5 49	5 84	5 95
	D_2O	60	3 56	7 20	7 36	5 29	5 74	6 06	6 12
	C_5D_5N–D_2O	23	3 12	6 95	7 12	4 84	5 36	5 79	5 84
	C_5D_5N–D_2O	0	3 07	6 86	7 07	4 76	5 31	5 76	5 82
Adenosine-*P*[b]	D_2O	28	3 04	6 86	7 05				
Guanosine (2)	Me_2SO	80	3 66	7 34	7 62	4 98	5 92	6 20	6 25
	Me_2SO	23	3 62	7 35	7 53	5 32	5 91	6 20	6 25
Guanosine-*P*	D_2O	80	3 70	7 22	7 43	5 28	5 70	5 97	6 02
	D_2O	23	3 77	7 22	7 43	5 28	5 70	5 93	5 98
Inosine (3)	Me_2SO	80	3 64	7 35	7 63	5 56	6 07	6 35	6 40
	Me_2SO	23	3 63	7 26	7 63	5 56	6 08	6 35	6 42
Pyrimidine derivatives									
Uridine (4)	C_5D_5N	80	3 24	7 52	7 37	5 04	5 64	5 90	5 95
	C_5D_5N	23	3 06	7 39	7 27	4 98	5 49	5 78	5 86
	C_5D_5N–D_2O	23	3 38	7 52	7 27	5 06	5 54	5 80	5 85
	C_5D_5N	0	2 98	7 36	7 24	4 94	5 46	5 74	5 82
	C_5D_5N	−40	2 83	7 26	7 18	4 84	5 37	5 69	5 77
Thymidine (5)	C_5D_5N	80	3 18	7 46	7 38	5 12	5 66	5 88	5 94
	C_5D_5N	23	2 97	7 35	7 28	4 95	5 52	5 76	5 84
Cytidine HCl[c] (6)	D_2O	80	3 93	7 76	7 64	5 74	6 06	6 31	6 38
	D_2O	23	3 92	7 78	7 68	5 82	6 08	6 35	6 40

[a]Chemical shifts in non-aqueous solvent are given relative to Me_4Si, otherwise shifts are given relative to DSS (sodium 4,4-dimethyl-4-silapentane-1-sulfonate) [b]See Ref 1 [c]These chemical shifts are given relative to the OH peak of the solvent, which was assigned a value of τ 4 38

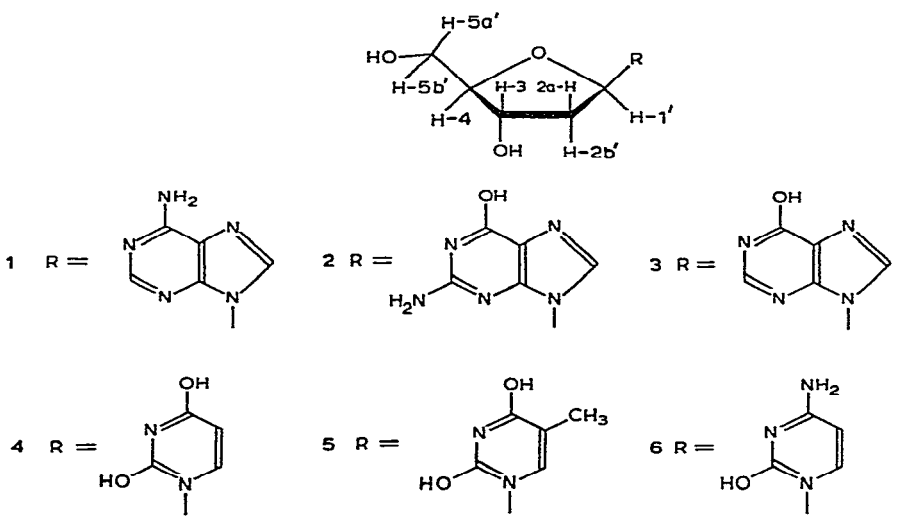

Fig 1 Structures of nucleosides studied 2'-Deoxyadenosine (1), 2'-deoxyguanosine (2), 2'-deoxyinosine (3), 2'-deoxyuridine (4), thymidine (5), 2'-deoxycytidine (6)

TABLE II

COUPLING CONSTANTS[a]

Compound	*Solvent*	*Temp (deg)*	$J_{1',2'a}$	$J_{1',2'b}$	$J_{1',3'}$	$J_{2'a,2'b}$	$J_{2'a,3'}$	$J_{2'b,3'}$	$J_{3',4'}$	$J_{4',5'a}$	$J_{4',5'b}$	$J_{5'a,5'b}$
Purine derivatives												
Adenosine (1)	C_5D_5N	80	7 28	6 23	—	−13 23	6 04	3 32	2 92	3 47	3 69	−11 95
	D_2O	60	7 27	6 31	—	−13 96	6 39	3 51	2 87	3 46	4 72	−12 51
	C_5D_5N-D_2O	23	7 41	6 03	—	−13 30	5 76	2 81	2 62	3 23	3 30	−12.25
	C_5D_5N-D_2O	0	7 40	5 98	—	−13 21	5 78	2 61	2 49	3 07	3 09	−12 29
Adenosine-*P*[b]	D_2O	28	6 9	6 6	—		5 8	3 8				
Guanosine (2)	Me_2SO	80	7 50	6 19	—	−13 20	6 04	3 31	3 00	4 37	4 31	−11 62
	Me_2SO	23	7 59	6 04	—	−13 22	5 74	2 96	2 77	4 44	4 17	−11 66
Guanosine *P*	D_2O	80	6 95	6 41	—	−13 82	6 43	3 98	3 46	5 93	5 29	− 8 32
		23	7 13	6 18	—	−13 72	6 24	3 51	3 04	—	—	—
Inosine (3)	Me_2SO	80	7 10	6 26	|0 46|	−13 31	6 05	3 49	3 15	4 42	4.44	−11 80
	Me_2SO	23	7 19	6 14	—	−13 18	6 00	3 26	2 62	4 62	4 39	−11 77
Pyrimidine derivatives												
Uridine (4)	C_5D_5N	80	6 80	6 33	|0 49|	−13 33	6 45	3 83	3 54	3 67	3 55	−11 78
	C_5D_5N	23	6 98	6 14	—	−13 15	6 06	3 68	3 28	3 29	3 05	−11 71
	C_5D_5N-D_2O	23	7 09	6 20	—	−13 52	6 28	3,62	3 40	3 48	3 61	−12 25
	C_5D_5N	0	6 88	6 07	—	−13 00	6 05	3 59	3 27	3 26	2 97	−11 88
	C_5D_5N[c]	−40	7 3	6 3	—	−13 2	6 5	3 4	2 1	3 0	2 4	−11 9
Thymidine (5)	C_5D_5N	80	6 86	6 32	|0 50|	−13 38	6 51	3 79	3 57	3 58	3 50	−11 86
	C_5D_5N	23	7 07	6 15	—	−13 11	6 17	3 68	3 01	3 08	3 08	−11 81
Cytidine·HCl (6)	D_2O	80	6 38	6 51	|0 49|	−14 21	6 62	4 26	4 02	3 77	5 13	−12 29
	D_2O	23	6 41	6 46	—	−14 14	6 55	4 26	3 95	3 53	5 00	−12 39

[a]Estimated error in $J = \pm 0\,1$ Hz [b]See Ref 1 [c]Care must be taken in use of these couplings since the peaks were broad and undefined, consequently very large errors could be present in some of the couplings

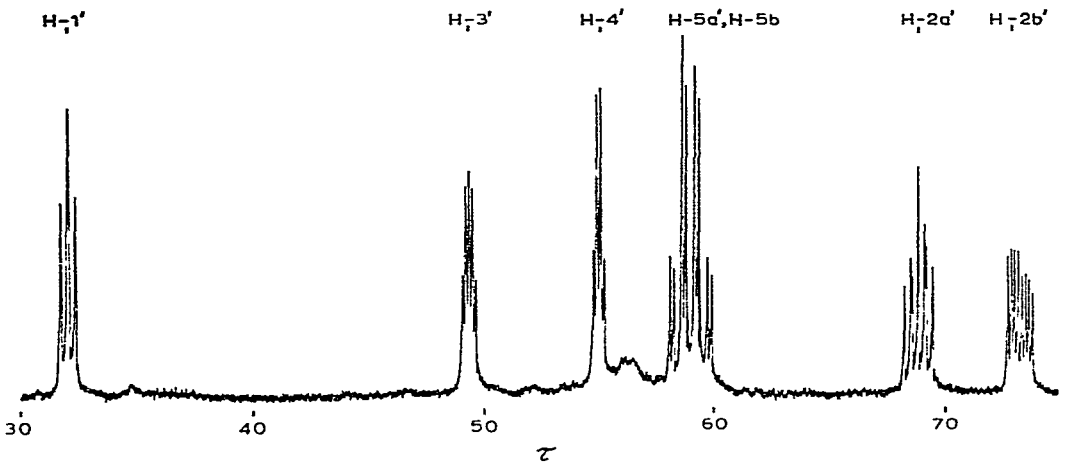

Fig 2 Representative 220-MHz spectrum of the deoxypentofuranosyl portion of 2′-deoxyadenosine (1) measured in pyridine-d_5 at 80°

temperatures, the resonances broadened considerably, making accurate analysis difficult The spectra obtained at −40° were almost completely unanalyzable, although limited information was obtained for 2′-deoxyuridine at this temperature Insolubility of certain of the compounds precluded obtaining spectra from D_2O solutions for all examples It was found, however, that no very significant differences in spectra were obtained on changing from aqueous to non-aqueous solution

Line broadening has been ascribed to base-stacking in aqueous systems[5] The presence of broadening in non-aqueous media, such as pyridine, in our opinion cannot be attributed to this type of interaction, as the base should be strongly solvated Thus, the characteristic broadening of the spectra at lower temperature indicates that conformational equilibration between various favored conformations is occurring As this broadening is quite evident even at room temperature, it would seem that the energy barrier to interconversion between various energy minima is relatively high In an equilibrating system, time-averaged couplings are observed If the deoxypentofuranose ring has a conformation that has an energy minimum significantly lower than that for any other conformation, this could be expected to be revealed by a temperature dependence in the interproton couplings As the temperature is lowered the observed couplings should tend toward those exhibited by a favored conformer, if one in fact exists The effect of dihedral-angle estimation by the ratio method (DAERM)[3] would be to yield averaged angles from these various sets of time-averaged coupling constants Through extrapolation of these changes it is possible to assign conformational tendencies to the molecule It should be noted that the dihedral angles that are exhibited in the assigned favored conformation may be very different from those obtained from the DAERM calculations The criterion is that contributions from alternative conformers will, by averaging, give the observed results

Dihedral Angle Estimation by the Ratio Method (DAERM) is a process of

estimating the angles subtended by a hydrogen atom coupled to a methylene group DAERM is based on the assumption that the $\cos^2\theta$ term of the Karplus equation is not significantly affected by various atomic and molecular parameters such as ring size and electronegativity In order to correlate change in couplings with conformational tendency, DAERM calculations were carried out for the H-1′ and H-3′ couplings into the methylene group at C-2′ As the results for all compounds except, perhaps, 2′-deoxycytidine HCl, were similar, only the results for 2′-deoxyadenosine are discussed in detail, and those results obtained for the other compounds are listed in Table III

TABLE III
DAERM ANGLES

Compound	*Temp (deg)*	*1′,2′* cis	*1′,2′* trans	*2′,3′* cis	*2′,3′* trans	$J_{3,4}$
Adenosine (1)	80	26	150	8	132	2 92
	60	26	150	8	132	2 87
	23	27	151	6	130	2 62
	0	27	152	4	128	2 49
Adenosine-*P*[a]	23	21	145	12	136	
Guanosine (2)	80	27	151	8	132	3 00
	23	29	153	7	131	2 77
Guanosine-*P*	80	24	148	11	135	3 46
	23	26	150	8	132	3 04
Inosine (3)	80	25	149	9	133	3 15
	23	26	150	8	132	2 62
Uridine (4)	80	24	148	9	133	3 54
	23 (C_5D_5N)	25	148	10	134	3 28
	23 (C_5D_5N–D_2O)	25	149	9	133	3 40
	0	25	149	10	134	3 27
	−40[b]	26	150	7	131	2 14
Thymidine (5)	80	25	149	9	133	3 57
	23	26	150	10	134	3 01
Cytidine HCl (6)	80	22	146	11	135	4 01
	23	22	146	12	136	3 95

[a]See ref 1 [b]See footnote *a*, Table II

From the high-temperature (80°) results, DAERM* provides two acceptable solutions (*i* and *ii*) for the H-1′ to H-2′ couplings Similarly the coupling of H-3′ into the methylene position provides two acceptable DAERM* solutions (*iii* and *iv*)

	J cis (*Hz*)	J trans (*Hz*)	cis *angle* (*deg*)	trans *angle* (*deg*)
i	7 28	6 23	18	142
ii	6 23	7 28	26	150
iii	6 04	3 32	8	132
iv	3 32	6 04	37	161

*$\omega = 124°$, $k_1/k_2 = 0\ 8$, values typical for furanose systems[2]

The configuration of the β-D-*erythro* system, and the couplings assigned in Table II allow only two combinations of these values (Fig 3)

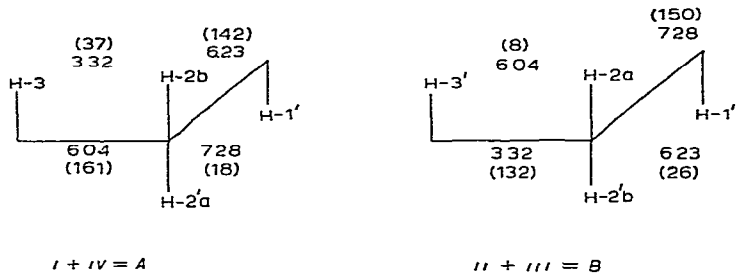

Fig 3 Two shapes of the 2'-deoxy-D-*erythro*-pentofuranosyl moiety (couplings in Hz, angles in degrees)

Studies[1] on the 3'- and 5'-phosphates of 2'-deoxyadenosine have indicated that the *trans*-coupling of protons between positions 1' and 2' is the larger, and consequently, the *trans*-coupling between positions 2' and 3' is the smaller This leads us to assign *B* as the correct conformational indicator, and thus the set of angles that most closely reflect the conformational averaging of the molecule This assignment is substantiated by the temperature dependence of the coupling constants (Table IV) In all of the compounds, the coupling between H-3' and H-4' decreases with decreasing temperature

TABLE IV

TEMPERATURE DEPENDENCE OF TIME-AVERAGED DIHEDRAL ANGLES

Temp (deg)	*1',2* cis	*1',2'* trans	*2',3'* cis	*2,'3* trans	$J_{3,4}$
Assignment A					
80	18	142	37	161	2 92
60	18	142	37	161	2 87
23	17	141	40	164	2 62
0	17	141	42	166	2 49
Assignment B					
80	26	150	8	132	2 92
60	26	150	8	132	2 87
23	27	151	5	130	2 62
0	28	152	4	128	2 49

The conformational tendency indicated by assignment *A* necessitates a conformer in which H-3' and H-4' are near *trans*-antiparallel Not only is $J_{3',4'}$ much smaller than would be expected for such an arrangement, but the relationships expressed by the temperature dependence of *A* would require an increase in the averaged H-3' to H-4' dihedral angle, and consequently an increase in the coupling

with decreasing temperature Clearly these observations are not in keeping with assignment *A*

The trends observed with decreasing temperature in assignment *B* (Table IV), dictate a decrease in the H-3′ to H-4′ dihedral angle to nearer 90°, and consequently a decrease in coupling Thus arrangement *B* agrees with the proton assignment of Ts'o and coworkers[1] and provides a reasonable basis for the temperature dependence of the H-3′–H-4′ coupling

Further examination of Tables III and IV indicates a slight, but systematic, increase in calculated *cis* (and *trans*) H-1′ to H-2′ dihedral angle as the temperature is lowered A similar trend is noted for the H-2′ to H-3′ angles, but the angles here collapse The change is much more rapid for this latter case and this, in conjunction with the corresponding decrease in the H-3′ to H-4′ coupling, indicates that the most mobile part of the molecule is that involving carbon atoms 3′ and 4′

The preceeding information on the couplings into C-2′ dictates that the favored conformation must be one in which this atom is above the average plane of the other four ring-atoms The conformational preference ${}^2V \rightleftarrows {}^2T_3 \rightleftarrows V_3$ as designated by Hall and coworkers[6] most closely approximates the direction in which the lower-temperature results show change With minimal change in angles about carbon atoms 1′–3′, the alternative favored conformer (${}^0V \rightleftarrows {}^0T_4 \rightleftarrows V_4$) can be achieved, in accordance with the higher-temperature results showing most change in the C-3′ to C-4′ region The favored conformation assigned is not surprising, in that ribose and deoxy-*erythro*-pentose nucleosides and nucleotides in the crystalline state often adopt the ${}^2V$ (C-2′ endo) and V_3 (C-3′ exo) conformations[7–9] The involvement of one or more hydrogen bonds within the crystalline species may obviously distort or change the conformation from that found in solution

2′-Deoxycytidine HCl, as mentioned previously, seems at first sight to be an exception It did not show a marked broadening at 23° (that is, the peaks were still quite sharp, although the 0 5-Hz coupling was no longer clearly resolved) The couplings in the furanose ring did not change noticeably with temperature, the maximum difference in couplings was 0 07 Hz, a value within the experimental error of ~0 1 Hz for this compound at 23° The H-3′ to H-4′ (and H-2′ to H-3′ trans) couplings are the largest observed in this study (approx 4 Hz) These results are readily explained if the difference in energy between the favored and alternative favored conformer is less in the case of cytidine than for other derivatives If this is true, more time will be spent in the alternative favored conformer, the H-3′ to H-4′ (and H-2′ to H-3′ trans) couplings will consequently be larger than that observed for the other compounds The equilibrium population will not be significantly changed with temperature (thus explaining the slight temperature-dependence of couplings) and the rate of interconversion between conformers will be faster and thus the n m r line-broadening will be less at 23° It should be noted that, in general, all of the pyrimidine derivatives show a slightly less-marked dependence of couplings on temperature than do the purine derivatives The H-3′ to H-4′ coupling is also invariably larger than for the purine case This would indicate that the purine

derivatives studied favor slightly more the ${}^2V \rightleftarrows {}^2T_3 \rightleftarrows V_3$ conformation than do the corresponding pyrimidine derivatives

In general, it was found that the chemical shifts of the H-2′ protons of the 5′-phosphate derivatives were so similar that good analysis of the spectra was impossible, and therefore little definitive information could be obtained concerning the temperature dependence of the couplings The spectrum of 2′-deoxyguanosine 5′-phosphate was readily analysed, however, and the temperature dependence of couplings was found similar to that of the other derivatives (Table II). As the couplings of the 2′-deoxyadenosine 5′-phosphate at room temperature[1] are similar to those of the derivatives that we have studied, it seems unlikely that this compound is an exception The foregoing compounds are both purine derivatives, and consequently little can be said about the conformational tendencies of the pyrimidine 5′-phosphate derivatives

EXPERIMENTAL

The 2′-deoxy derivatives were purchased from Sigma Chemical Co and recrystallized twice from D_2O or D_2O–pyridine Variable temperature n m r studies were conducted on the Canadian Research Council's 220-MHz spectrometer at Sheridan Park, Ontario, Canada Spectral analysis was performed by using an IBM 370/155 computer and the LAOCN III program of Bothner-By and Castellano[4] Chemical shifts in non-aqueous solvent were measured relative to Me_4Si, in D_2O or solvent mixtures, relative to DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid, sodium salt)

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