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CONFORMATIONAL ANALYSIS OF 2′,3′-O-ISOPROPYLIDENE-ADENOSINE-5′-ACETATE IN ORGANIC SOLVENTS BY QUANTITATIVE APPLICATION OF THE NUCLEAR OVERHAUSER EFFECT

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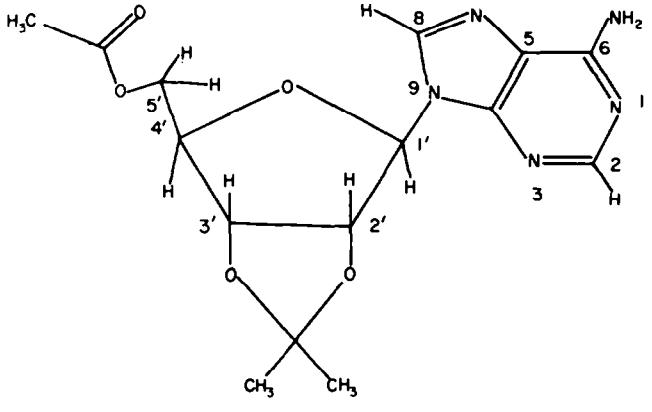
Abstract—The conformation of 2′,3′-isopropylidene-adenosine-5′-acetate in several organic solvents is studied by quantitative application of the nuclear Overhauser effect. Solvent and temperature effects on nucleoside proton chemical shifts are discussed.

INTRODUCTION

THE CALCULATION of proton nuclear Overhauser effects (NOE's) in a multispin system as a function of molecular conformation and the determination of glycosyl conformer distributions by computer fit of multiple experimental intramolecular NOE's has recently been described.[1] We describe here the conformational analysis of 2′,3′-isopropylidene-adenosine-5′-acetate (I) in organic solvents on the basis of quantitative nuclear Overhauser effects, along with chemical shift, and spin coupling constant data.

RESULTS AND DISCUSSION

Conformational fits (Fig 5) of the NOE data at 31° (Table 1) put the adenine N-3 in the region of H-2′. The incomplete variable temperature NOE's (Table 2) measured



I

FIG 1. 2′,3′-O-isopropylidene-adenosine-5′-acetate (I)

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on H-8, the reporter proton most sensitive to glycosyl conformation, indicate little or no change in glycosyl conformation from $+50°$ to $-50°$. Over a smaller range of temperature, 30° to 60°, Ts'o *et al.*[6] concluded that there is no change in the average sugar-base torsion angle of purine 5'- and 3'-nucleotides in water.

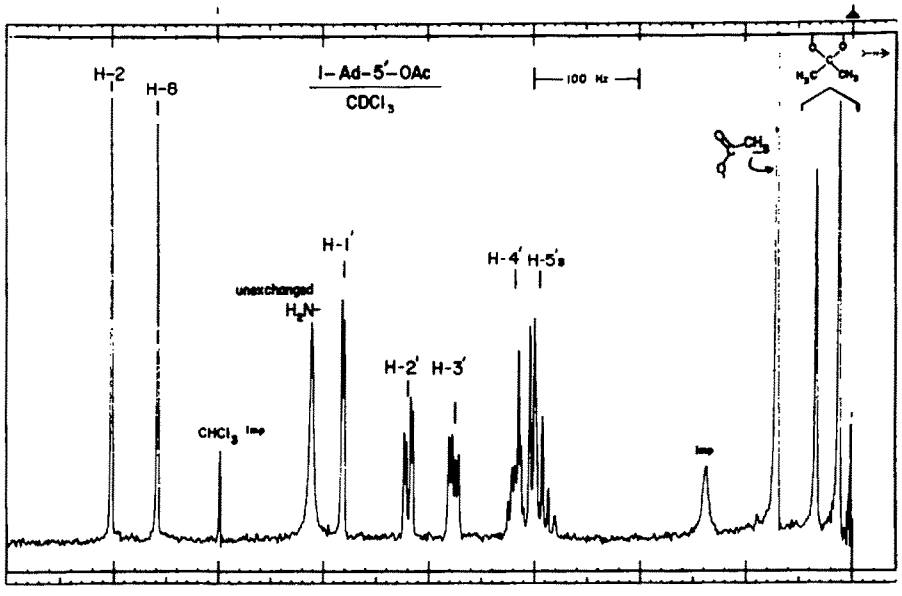

FIG 2. 100 MHz NMR spectrum of 2',3'-O-isopropylidene-adenosine-5'-acetate, 0·25 M in $CDCl_3$

Ribose proton chemical shifts as a function of solvent (Table 3) do not appear informative. The ribose proton chemical shifts (Fig 6) exhibit small but distinct changes with temperature. Excluding $J_{4'-5',5''}$, the ribose coupling constants (Table 3)

TABLE 1. NUCLEAR OVERHAUSER EFFECTS IN *I*[a]

Solvent	$f_s(1')$	$f_s(2')$	$f_s(3')$
DMSO-d_6	0·21	0·04	0·0
acetone-d_6	0·24	0·08	0·02
$CDCl_3$	0·22	0·02	0·0

[a] 0·25 M, 31°

vary less than 0·5 Hz from $+50°$ to $-50°$, implying that a ribose conformational change is not responsible for the observed chemical shift trends with changing temperature. The H-4'–5',5'' coupling constants appear to vary more than one Hz over the same range, but were not measured precisely because of the complexity of that region.

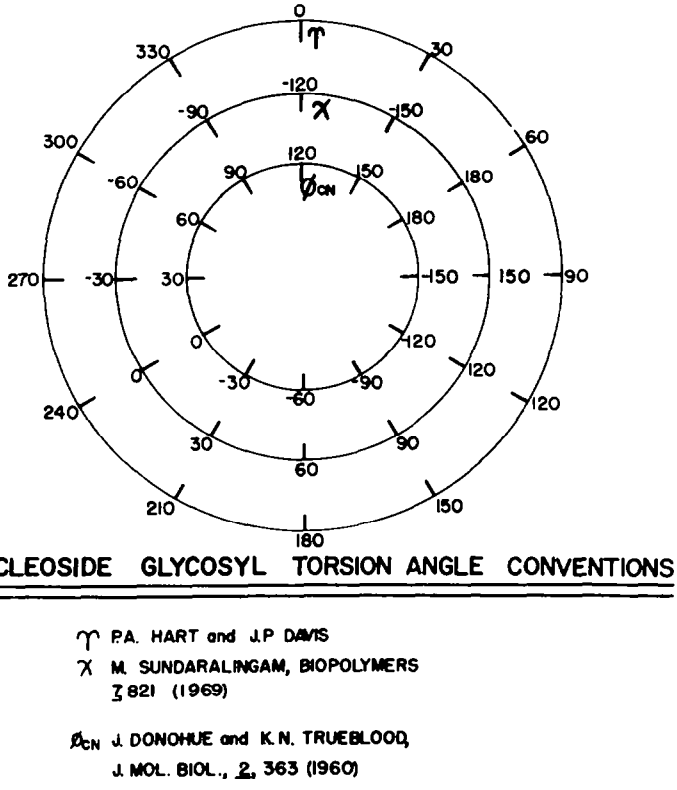

FIG 3. Nucleoside glycosyl torsion angle conventions

The H-2′ resonance of I in both acetone and $CDCl_3$ shifts slightly upfield with decreasing temperature while the other ribose resonances, except H-5′,5″ in acetone, move downfield. With decreasing temperature, it is reasonable to expect the (preferred) conformational distributions in I to narrow, yielding a greater proportion of conformers in which the pyrimidine portion of adenine is close to H-2′ and a smaller proportion of others. The shielding of H-2′ with decreasing temperature is probably

TABLE 2. TEMPERATURE INFLUENCE ON NUCLEAR OVERHAUSER EFFECTS IN I[a]

Solvent	Temp.	$f_8(1')$
acetone-d_6	+50	0·24
	+30	0·25
	−30	0·28
$CDCl_3$	+50	0·24
	+30	0·20
	0·0	0·21

[a] 0·16 M

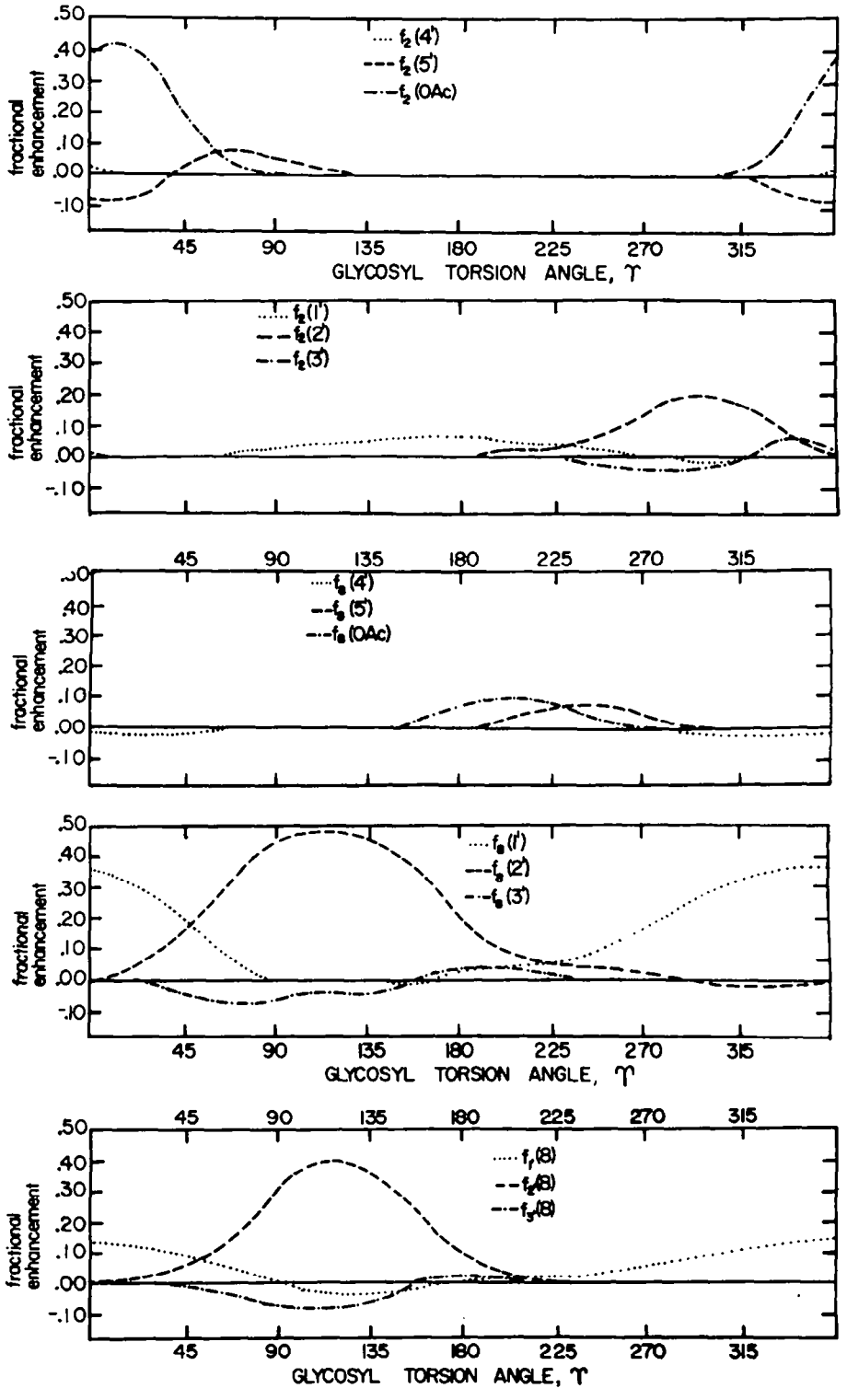

FIG 4. Plots of calculated intramolecular nuclear Overhauser effects. C-3' endo, C-4' exo ribose geometry. Region I conformer exchange rate. External relaxation parameter value equals 0·0005. See experimental section for details

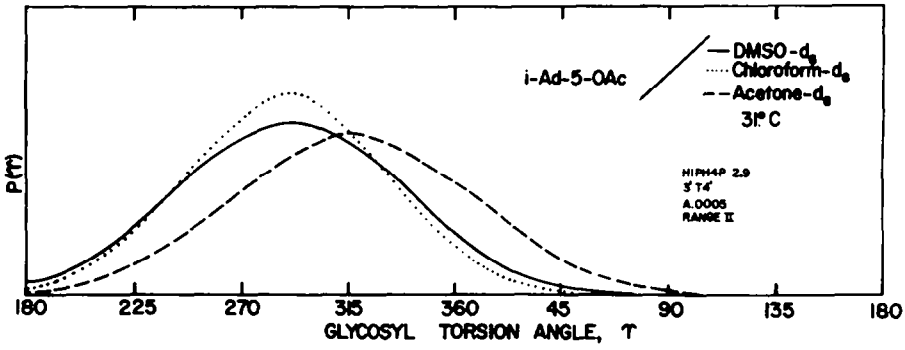

FIG 5. Computer fits of glycosyl conformation to experimental nuclear Overhauser effects in Table 1. The entire fitting procedure was carried out for glycosyl conformer interconversion rates in Region II (see experimental section)

not due to the electronic field effect of the (largely delocalized) lone pair of the nearby N-3. By analogy with the effect of an ether oxygen[7] this would result in a downfield shift of H-2'. The ring current supported by the pyrimidine portion of the purine base is a possible cause of the H-2' shielding. Such a mechanism is reasonable here since in the derived glycosyl conformation H-2' is on the outer edge of the typical shielding region[8a,b] of the aromatic pyrimidine portion of the purine base.

In order to gain information on the disposition of the 5' acetate with respect to the adenine moiety the nucleoside acetate methyl chemical shifts were compared with the chemical shifts of the acetate methyl of 0·25 M EtOAc in the respective solvents (Table 5). If the 5' acetate were associated to the pyrimidine portion of the nucleobase its chemical shift would be shifted by 0·1 ppm or more,[8b] either upfield or downfield depending on the precise geometry of the association, from the standard ethyl acetate methyl resonance. Such a chemical shift difference is observed only in MeOH, the effects of which may be complicated by the solvent's ability to both donate and accept H-bonds. Few further NMR data are available in MeOH Chemical shift differences in the remaining solvents are small. Non-bonded interaction energy calculations have suggested that the C4', C5' conformation exerts a minor influence on nucleoside glycosyl torsion angle.[9]

TABLE 3. RIBOSE CHEMICAL SHIFTS AND SPIN COUPLING CONSTANTS OF I[a]

Solvent	δ_{TMS}					$J_{(Hz)}$		
	H-1'	H-2'	H-3'	H-4'	H-5',5''	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$
DMSO-d_6	6·18	5·47	5·04	4·33	4·17	2·6	6·3	3·3
CD_3CN	6·16	5·47	5·04	4·44	4·23	2·4	6·1	3·0
acetone-d_6	6·23	5·57	5·13	4·41	4·27	2·0	6·0	3·2
CD_3OD	6·24	5·56	5·12	4·47	4·29	2·5	6·2	3·1
$CDCl_3$	6·07	5·46	5·02	4·43	4·26	2·0	6·0	3·5

[a] 0·25 M nucleoside, 31°

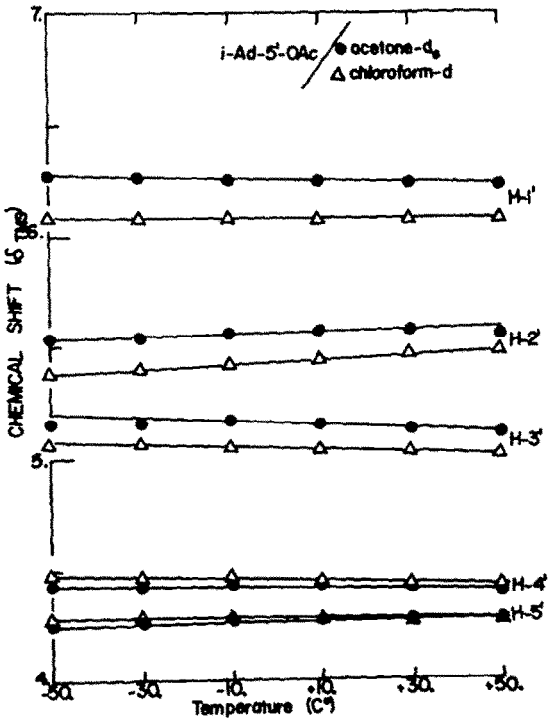

FIG 6. Temperature effects on the ribose proton chemical shifts of I

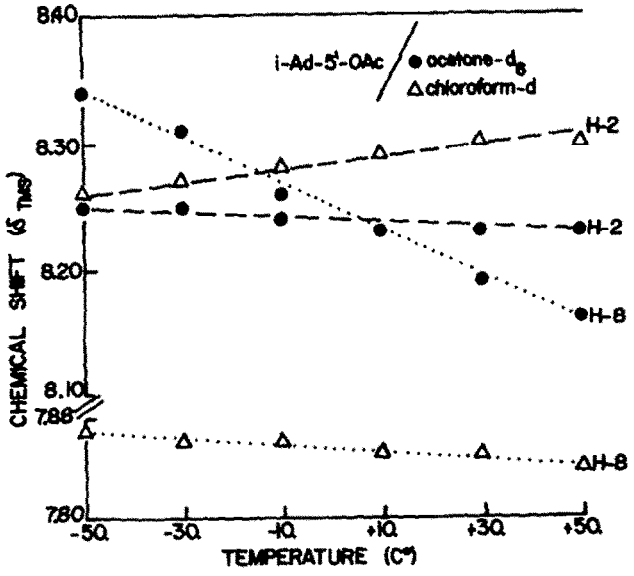

FIG 7. Temperature effects on the nucleobase proton chemical shifts of I

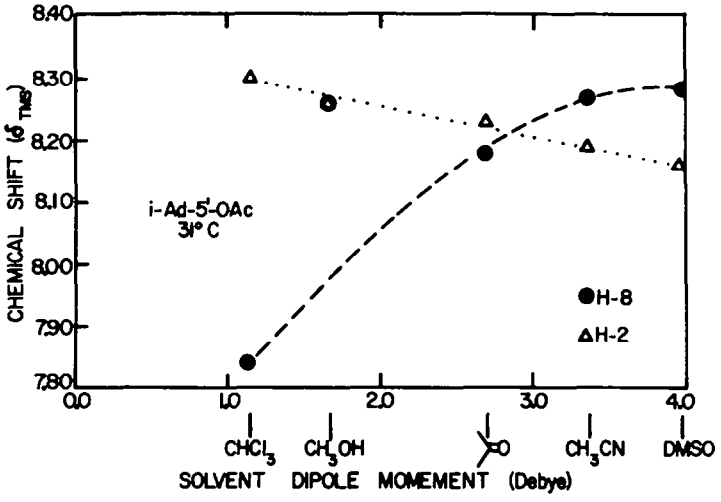

FIG 8. Correlation of nucleobase proton chemical shifts with solvent dipole moment

Temperature and solvent dependence of the nucleobase proton chemical shifts

The shift of H-8 with temperature (Fig 7) appears to be due to the lability or "acidity" of that proton. This behavior has been documented by Hruska *et al.*,[10] among other groups, and discussed by Ts'o.[11] The purine protons, H-2 and H-6 when present, are not labile by comparison. Thus, the decrease of $\delta_{H\text{-}8}$ in acetone with increasing temperature reflects a decrease in H-8 hydrogen bonding to solvent as the thermal energy of the system is increased. $\delta_{H\text{-}8}$ has a small negative slope with temperature in $CHCl_3$. This solvent acts exclusively as a hydrogen bond donor and there is probably little specific interaction of H-8 with solvent in this case. The slight positive slope of $\delta_{H\text{-}2}$ with temperature may imply a decrease in some specific interaction of $CHCl_3$ with the pyrimidine moiety which normally causes relative shielding of H-2.

Intramolecular hydrogen bonding of H-8 with the ribose ring oxygen may also explain observed temperature variation of $\delta_{H\text{-}8}$ though the strength of such an

TABLE 4. CHEMICAL SHIFTS OF ACETATE METHYL GROUPS

δ_{TMS}

Solvent	Acetate methyl of I[a]	Acetate methyl of ethyl acetate[a]
DMSO-d_6	2·00	2·03
CD_3CN	1·96	1·99
$CD_3\overset{O}{\overset{\|}{C}}—CD_3$	1·99	1·99
CD_3OD	1·98	2·04
$CDCl_3$	1·97	1·98

[a] 0·25 M, 31°

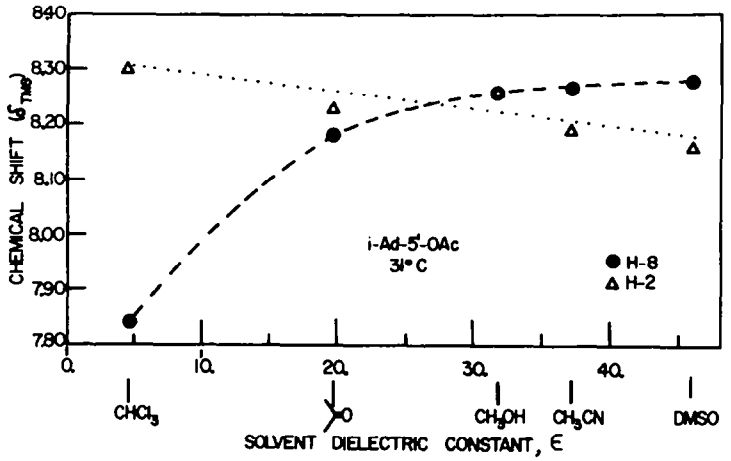

FIG 9. Correlation of nucleobase proton chemical shifts with solvent dielectric constant

interaction is apt to be small due to the deviation of the overlapping orbitals from linearity.[12] It is difficult to distinguish this mechanism from hydrogen bonding with solvent in the case of the hydrogen bond acceptors, acetone and DMSO. Nucleobase proton chemical shifts are correlated with solvent dipole moment and dielectric constant in Figs 8, 9. Values in MeOH fall out of line with the rest because of the bifunctional character of the alcohol.

Other mechanisms, for example a simple reaction field theory,[13] might also explain the magnitude, direction, temperature dependence, and solvent dielectric dependence of the observed H-8 and H-2 chemical shifts. To rigorously specify the mechanism of the observed variations, further, meticulous, experimentation would be required.

UV and CD spectra

Except for their unusual broadness and a slight shoulder on the short wavelength side of the envelope the nearly symmetric long wavelength UV absorption curves (Fig 10, Table 5) give no indication of multiple transitions. The typical $B_{1u}^{\pi-\pi^*}$ transition of adenosine nucleosides[14] is conspicuously absent from its normal position ($\approx$240 nm) in the UV spectra of I. The CD spectra of I (Fig 9) suggest the presence of two transitions in the region of 250 to 265 nm.

TABLE 5. UV ABSORPTION CONSTANTS OF *I*[a]

Solvent	λ_{max}	ϵ_{max}
DMSO	262	14,630
CH_3CN	258	15,170
MeOH	259	15,000
$CHCl_3$	259·5	13,500

[a] 6×10^{-5} M, 25°

The major one is certainly the B_{2u} band at approximately 260 nm, and the minor one, which apparently has a maximum positive ellipticity at about 254 nm, could be the B_{1u} transition at an uncommonly long wavelength, or an n–π^* transition. It is probably not an n–π^* band since its UV characteristics (Table 5) would then be markedly perturbed by the various solvents employed. Previous subtle indications of a secondary transition in the 260 nm envelope have been discussed.[14]

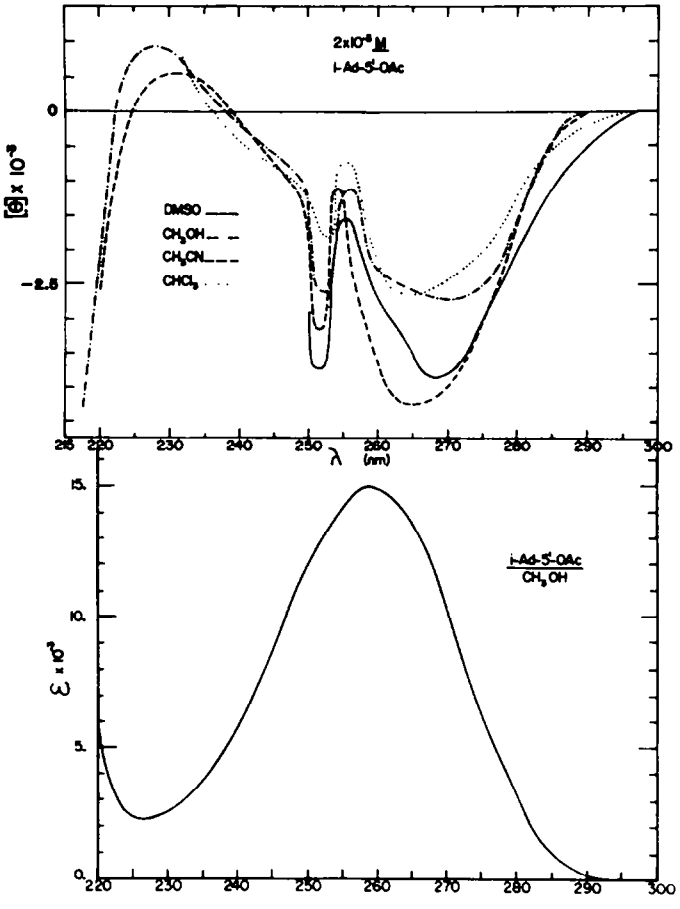

FIG 10. Circular dichroism spectra of I along with a typical UV spectrum

Miles *et al.*[15] calculated a positive cotton effect for the B_{2u} transition of C-3' endo adenosine in the same glycosyl conformation as that of I determined here by NMR ($\gamma \approx 290°$). The long wavelength ellipticity of I is negative in four organic solvents, three of which served in the NMR experiments (Fig 9).

Purine nucleoside glycosyl conformations have been predicted from theoretical considerations. A leading reference to such results is that of Berthod and Pullman.[16] The general conclusion for unsolvated purine nucleoside models is that the *anti* glycosyl conformation is favored over the *syn* by 1 to 2 kcal/mole. It is difficult to

point to a specific external force which would support a purine nucleoside glycosyl conformation such as found here for I. Solution considerations which might mitigate theoretical predictions are presented in ref. 1.

Since the calculated barriers to interconversion between the *syn* and *anti* conformations of purine nucleosides are small, subtle effects can alter the glycosyl conformation. The incongruence of the present results and theoretical predictions of nucleoside conformation suggests that interaction with the environment may be the ultimate determinant of nucleoside solution conformation.

CONCLUSION

Computer fitting of multiple intramolecular nuclear Overhauser effects is shown to be a sensitive means of quantitatively determining the conformation of small molecules in solution. Thus, the shape of small organic chemical messengers, both genetic and pharmacological, can be precisely determined in the liquid phase. Also, solvent- and temperature-dependent physicochemical parameters, e.g. chemical shift, optical spectra, etc., can be studied knowing the extent to which the observed measurements might be mediated by conformational changes in the species studied.

EXPERIMENTAL

2′,3′-isopropylidene-adenosine-5′-acetate was purchased from Sigma. Acetone-d_6 (99%d) and DMSO-d_6 (99·5%d) were products of Diaprep. $CDCl_3$ (99·8%d), methanol-d_4 (99·5%d) and t-BuOD were purchased from Merck. Acetonitrile-d_3 (99%d). EtOAc was Merck Reagent grade. Solvents used in the optical experiments were purified as described previously.[3] Lock sample (t-BuOD) concentration was 1·8 to 1·9% v/v before mixing. NOE samples (0·25 M nucleoside) were made up in precision 5 mm OD NMR tubes and degassed by multiple freeze-pump-thaw cycles at less than 10^{-5} torr before being sealed.

Peak height NOE experiments were performed as previously described.[2,3] Except where noted, enhancements reported here are an average of three to five determinations. $f_x(y)$ denotes the fractional enhancement of resonance x upon saturation of resonance y. The standard deviation, σ, in $f_x(y)$ varies from 0·02 to 0·03 from experiment to experiment. This points up the fact that the major limitation to this type of experiment is present commercial NMR spectrometer precision.

Temperature studies of the chemical shifts were made on 0·16 M undegassed solutions in acetone-d_6 and in $CDCl_3$. The nucleoside in both cases was refluxed in D_2O for 2 hr to exchange deuterium for H-8 and lyophilized before the NMR solution was made. The greatly diminished H-8 resonance could then be identified unambiguously (Fig 2) throughout the variable temperature experiments. Even at 0·16 M I in MeOH and also in MeCN slowly precipitated at 30° after being heated slightly to achieve dissolution. Thus, conventional NMR spectra were obtainable but NOE experiments were precluded.

I was not studied in DMSO as a function of temperature because that sample could sustain a smaller temperature range; pure DMSO freezes at 18·4° and pure t-BuOH vaporizes at 82·8°.

Recorded NMR spectrometer probe temperatures are ±1·0°. All chemical shifts of I were invariant to within ±0·5 Hz and natural linewidths of about 1. Hz did not vary from 0·05 to 0·25 M nucleoside in pure $CDCl_3$ and pure acetone-d_6: the addition of 2·0% v/vt-BuOD did not effect the chemical shifts in either solvent.

CD measurements were made on a Cary 60 recording spectropolatimeter fitted with a Model 6002 CD attachment, with the slit programmed for a half-bandwidth of 15 Å. The low wavelength region of the spectrum was inaccessible for the DMSO solutions because of high solvent absorbance below *ca.* 250 nm. The CD is recorded as molecular ellipticity, $[\theta]$, in units of deg.cm^{2}dmol^{-1}, and absorbances did not exceed 3. The instrument was calibrated using (+)-camphor sulfonic acid (Aldrich). Nucleoside concentration for the CD experiments done in 0·1 cm cells was $2·0 \times 10^{-3}$ M in $CHCl_3$, DMSO, MeCN and MeOH. Acetone absorbance does not permit such measurements. UV spectra were recorded on a Cary 14 instrument.

We define the glycosyl torsion angle, upsilon (γ), as the angle between the plane of the nucleobase and the plane determined by the glycosyl bond and the C1′—H1′ bond. N9 is always taken to be sp^2 hybridized.

γ is taken as positive, running from 0·0°, with H-1' and X-8 eclipsed, to 360°. It is measured clockwise from X-1' to X-8 while sighting down the glycosyl bond from N-9 to C-1'. Further discussion of this definition can be found in Ref 1. The ϕ_{CN} and χ equivalents of γ are given in Fig 3.

The geometric parameters required for the NOE calculations were taken from Framework Molecular Models* of 2',3'-isopropyledene-adenosine-5'-acetate constructed using X-ray bond lengths and angles of adenosine.[4] The basic ribose conformation, suggested by the proton spin coupling constants, was C-3' *endo*, C-4' *exo*. The bulky substituents at C-1' and C-5' are thus pseudoequatorial. Of several different ribose ring geometries this one gave the best fit of observed NOE's. The fits of glycosyl torsion angle are insensitive within experimental error to the exact position of the acetate methyl provided it is in any one of a number of reasonable extended conformations (away from the nucleobase and C-5', see below).

The theoretical intramolecular enhancements plotted here as a function of glycosyl torsion angle (Fig 4) were calculated assuming an exchange rate for discrete glycosyl rotamers of 1 sec^{-1} or less ("Region I"). This was necessary since for the actual interconversion rate in such a system (probably on the order of 10^6 to 10^8 sec^{-1}, Region II) a calculational technique is necessary which precludes calculation of enhancements for discrete conformations.[1] However, experience has shown[5] that the enhancements calculated in Region I are illustrative of the effects occurring at realistic rates of conformer exchange ("Region II", k = 10^1 to 10^9 sec^{-1}). These plots demonstrate the unique sensitivity of nucleoside NOE's to the glycosyl torsion angle.

The conformational fits were performed in Region II. An external relaxation parameter,[1] A, value of 0·0005 was used.

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