Medium Effects on the Nuclear Magnetic Resonance Spectra of Purines*

F. E. Hruska, C. L. Bell,† T. A. Victor, and S. S. Danyluk

ABSTRACT: The proton chemical shifts of purine, 6-methylpurine, and 9-ribosylpurine have been studied at several concentrations in a series of solvents and binary solvent mixtures with varying proton acceptor strengths and dielectric constants. In nonaqueous media large variations in the chemical shift of the H-8 proton were noted, whereas the H-2 and H-6 (and methyl) protons were essentially solvent independent. The H-8 protons were shifted to low field in proton, acceptor solvents and in binary mixtures with increasing proton-acceptor concentration. The magnitudes of the shift changes are roughly correlated with the relative proton-acceptor strengths of the solvent. These results can be explained on the basis of a hydrogen-bonding interaction between H-8 and the proton acceptor group of the solvent molecule. In binary aqueous mixtures, on the other hand, the purine ring protons are initially shifted to low field with increasing D_2O concentration. At D_2O concentrations above 60 wt % the trend is reversed and the signals shift upfield with further D_2O addition.

The largest deshieldings are again observed for H-8 protons, while the largest shieldings are noted for H-2 and H-6 protons. These shift trends can be attributed to the combined effect of hydrogen-bonding (H-8 proton) and base-stacking interactions the influences of which are dominant at low and high D_2O concentrations, respectively.

Attention has been focused recently upon the nature of solute–solute and solute–solvent hydrogen-bonding interactions of purine and pyrimidine bases, nucleosides, and nucleotides in water and in a number of nonaqueous solvents ($CDCl_3$, DMSO, and DMF[1]). The results of extensive nuclear magnetic resonance (Katz and Penman, 1966; Shoup *et al.*, 1966), infrared (Kuchler and Derkosch, 1966; Hamlin *et al.*, 1965; Kyogoku *et al.*, 1961, 1966, 1967a,b), and ultraviolet (Thomas and Kyogoku, 1967) spectroscopic studies have shown that the hydrogen-bonding interaction between purine and pyrimidine bases, and nucleosides in $CDCl_3$, CCl_4, and DMSO is of the complementary Watson–Crick type. Infrared measurements (Kyogoku *et al.*, 1966, 1967a,b) indicate a self-association of purine and pyrimidine derivatives into hydrogen-bonded dimers in chloroform and confirm the presence of hydrogen bonds between appropriate proton accepting groups (*i.e.*, carbonyl, amino, and imino) on the bases and the solvent molecules (H_2O and DMSO). Furthermore, Raman studies (Lord and Thomas, 1967a,b) show no evidence of a complementary hydrogen-bonding interaction between purines and pyrimidines in aqueous solution.

In contrast with the detailed information available for the hydrogen-bonding properties of the carbonyl, amino, and imino groups on the bases relatively little is known about the proton donor properties of the C–H protons themselves. Molecular orbital calculations (Pullman and Pullman, 1963) and dipole moment measurements (DeVoe and Tinoco, 1962) indicate that the C-8 carbon is the most electron-deficient center in purines. These results, along with the facile base-catalyzed deuterium exchange of the H-8 proton (Shelton and Clark, 1967), suggest that the latter possesses some acidic character and may form hydrogen bonds under certain conditions. This possibility has been indicated by nuclear magnetic resonance studies (Katz and Penman, 1966) of nucleoside interactions in $CDCl_3$ and DMSO. In order to assess more fully the potential hydrogen-bonding properties of the ring C–H protons and the influence of such an interaction upon the stacking interaction of purine derivatives in aqueous solution, we have studied the proton chemical shifts for purine, 6-methylpurine, and 9-ribosylpurine in a wide variety of solvents and binary solvent mixtures with different dielectric strengths and hydrogen-bonding properties. The chemical shift data show that the H-8 proton of the purines can form hydrogen bonds both in aqueous and nonaqueous media. No tendency toward hydrogen-bond formation is noted for the H-2 and H-6 protons in nonaqueous solvents. The results also confirm that purine base stacking occurs to a significant extent in aqueous solutions only and that the base-stacking interaction is not primarily due to electric dipole–dipole forces.

Experimental Section

Materials. Purine, 6-methylpurine, and 9-ribosyl-

* From the Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois. *Received May 29, 1968.* Work supported by the U. S. Atomic Energy Commission.

† Visiting summer scientist, 1967. Permanent address: University of Illinois, at the Medical Center, Chicago, Ill. 60612.

[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: DMSO, dimethyl sulfoxide; DMF, *N,N*-dimethylformamide.

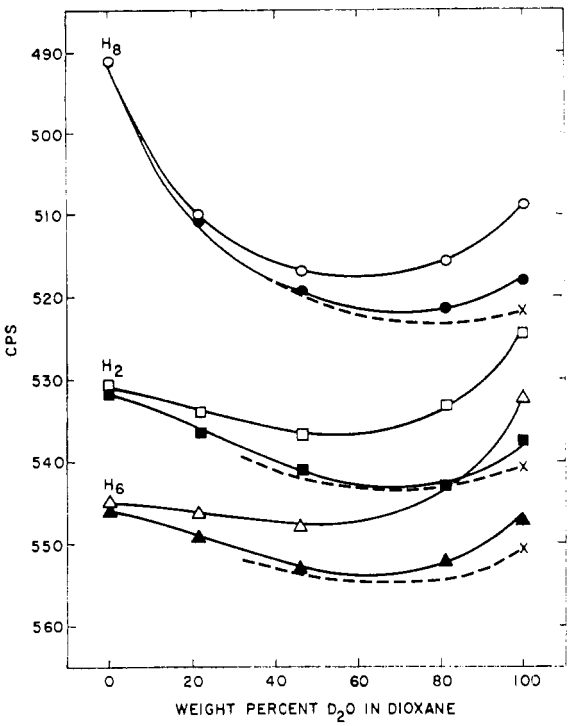


FIGURE 1: Proton chemical shifts of purine (at 32° relative to internal tetramethylsilane) *vs.* the weight per cent composition of aqueous dioxane solutions. Purine concentration: 0.50 M (open symbols), 0.05 M (solid symbols), and 0.0050 M (X).

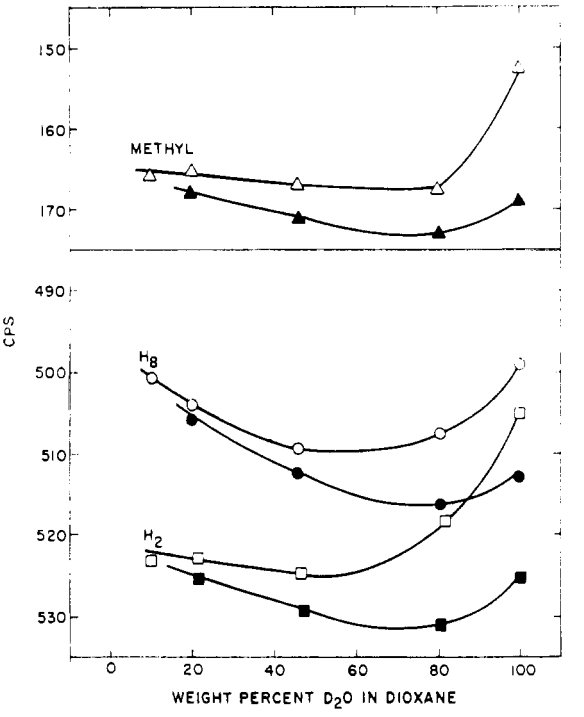


FIGURE 2: Proton chemical shift of 6-methylpurine (at 32° relative to internal tetramethylsilane) *vs.* the weight per cent composition of aqueous dioxane solutions. 6-Methylpurine concentration: 0.33 M (open symbols) and 0.05 M (solid symbols).

purine were purchased from Sigma Chemical, Calbiochem, and Aldrich Chemical Co., respectively. Dioxane and acetonitrile were purchased from Matheson Coleman and Bell while the other solvents were obtained from J. T. Baker Co. All of the chemicals and solvents were of the highest quality available commercially and were used without further purification. The D_2O was 99.8% isotopic purity and was supplied by the U. S. Atomic Energy Commission.

Preparation. Dioxane–water mixtures of known dielectric constant were prepared according to procedures described in the literature (Critchfield *et al.*, 1953). All other solutions were made up by weight to the desired concentration and were stored in stoppered vials in a desiccator until use. Because of solubility limitations purine was made up as a saturated solution in neat dixoane and chloroform.

Nuclear Magnetic Resonance Measurements. All of the proton spectra were recorded at ambient temperature (32 ± 1°) with a Varian DA-60 IL spectrometer operating in the internal-lock mode. For solutions in nonaqueous solvents and in binary solvent mixtures containing D_2O the spectrometer was locked on internal tetramethylsilane (~1–3%), while for solutions in D_2O, the spectrometer was locked to tetramethylsilane contained in a capillary immersed in the solution.

The chemical shifts reported for solutions with a solute concentration greater than 0.10 M represent an average of several scans on the 50-cps sweep-width range of the spectrometer. For solutions with a concentration lower than 0.10 M the signal-to-noise ratio was enhanced by repetitive scanning with a Varian C-1024 time-averaging computer. In each case the spectra were calibrated by measuring the difference between the sweep and lock frequencies with a Hewlett-Packard 5245L counter. Line positions were obtained by interpolation and are accurate to ±0.40 cps. All of the shifts are given relative to internal tetramethylsilane. The chemical shift of internal tetramethylsilane in D_2O solution was obtained by extrapolating the shifts in dioxane–D_2O mixtures to 0% dioxane. In addition, the measurements were made relative to an external capillary of tetramethylsilane and corrected for bulk susceptibility. Both methods exhibited the same chemical shift curve behavior with solvent and solute concentration.

Results

Dioxane–D_2O. Figures 1 and 2 illustrate the dependence of the ring proton shifts for purine and 6-methylpurine, respectively, upon solvent composition and solute concentration, while Figure 3 shows the corresponding behavior for 9-ribosylpurine. The signals are assigned in order of increasing field to the H-6, H-2, and H-8 protons for each purine base (Schweizer *et al.*, 1964; Bullock and Jardetzky, 1964).

All of the curves follow roughly the same pattern with increasing D_2O concentration. Addition of small amounts of D_2O results in an initial downfield shift for the ring protons with the largest rate of change observed for the H-8 proton. With further addition of D_2O the shifts level off and pass through minima in the

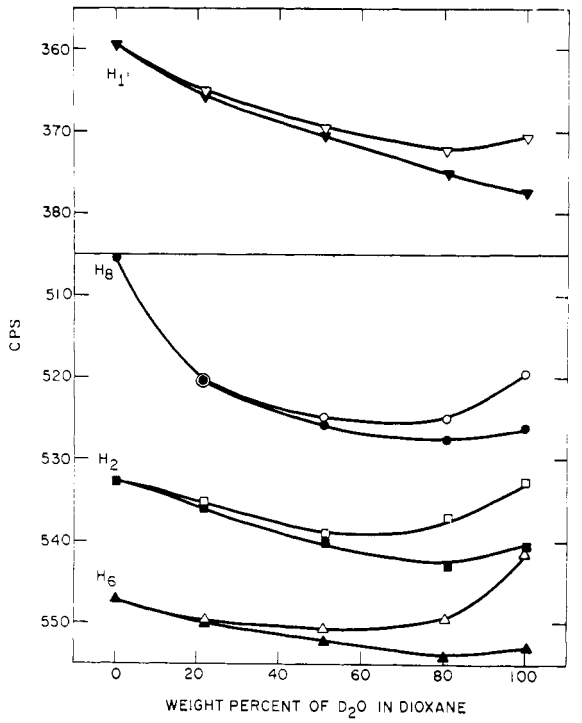


FIGURE 3: Proton chemical shifts of 9-ribosylpurine (at 32° relative internal tetramethylsilane) *vs.* weight per cent compositions of aqueous dioxane solutions. 9-Ribosylpurine concentration: 0.2 M (open symbols) and 0.02 M (solid symbols).

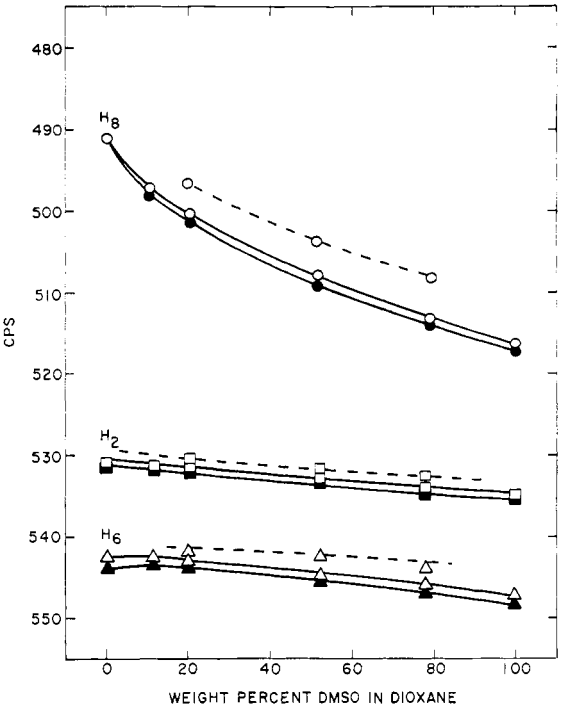


FIGURE 4: Proton chemical shifts of purine (relative to internal tetramethylsilane) *vs.* weight per cent composition of DMSO–dioxane solutions. Purine concentration: 0.2 M (open symbols) and 0.04 M (solid symbols). Temperature: (—) 32 and (— —) 77°.

concentration range 50–70 wt % D_2O. At D_2O concentrations higher than ~70 wt % upfield shifts are noted for all of the protons with increasing D_2O concentration. In this case, however, the shift changes from the minima to 100% D_2O are greater for the H-2 and H-6 (or methyl) protons than for the H-8 proton.

A pronounced solute concentration effect is observed for all of the compounds in binary solutions containing D_2O. In each instance the signals for the ring and methyl protons are located at lower field in more dilute solutions. This solute concentration effect is also related to the D_2O content of the mixture and becomes more noticeable with increasing D_2O. Again the largest changes are noted for the H-2 and H-6 (or methyl) protons.

Dioxane–DMSO. The effect of solvent composition upon the ring proton shift of purine, at two purine concentrations, is shown in Figure 4. A marked solvent dependence is noted for the H-8 proton with the shift decreasing by almost 25 cps in going from neat dioxane to neat DMSO. The H-2 and H-6 protons, on the other hand, show only a very slight shift to low field with increasing DMSO concentration, the over-all change amounting to less than 4 cps in each case. In contrast to the behavior in dioxane–D_2O solutions there is little if any dependence of the shifts upon the purine concentration over the entire binary solvent range, nor is there any evidence of minima in any of the curves. Also shown in Figure 4 are the purine shifts (0.20 M) in a number of dioxane–DMSO solutions at 77°. Although the shifts for the ring protons are located at somewhat higher field (2–5 cps) the curves follow the same pattern as at 32°. The H-8 proton again shows a larger change (~5 cps) than the H-2 and H-6 protons.

Figure 5 summarizes the dependence of the ring proton shifts[2] of 6-methylpurine upon solute concentration and solvent composition. As in the case of purine, the H-8 proton of 6-methylpurine shows a much more pronounced shift to low field with increasing DMSO concentration than the H-2 proton.

Other Binary Solvent Systems. The chemical shift dependence upon solvent composition is further illustrated for the purine protons in dioxane–nitromethane and dioxane–acetone (Figure 6), and in dioxane–acetonitrile mixtures (Figure 7). As in the case of the dioxane–DMSO system the H-2 and H-6 proton shifts are nearly independent of the composition of the binary mixture. Moreover, the shifts for these two protons are essentially the same in all four binary systems. The H-8 proton shifts, however, are strongly influenced by the nature of the second solvent in the mixture, shifting downfield by 6, 10, and 17 cps upon addition of acetonitrile, nitromethane, and acetone, respectively, to the dioxane.

A summary of the shifts for the purine protons in a variety of neat nonaqueous solvents is given in Table I. The solvents cover a range of dielectric properties and proton donor ($CDCl_3$) and acceptor (DMSO) abilities. Somewhat surprisingly, the purine signals are located

[2] The methyl proton signal is obscured by the solvent peak

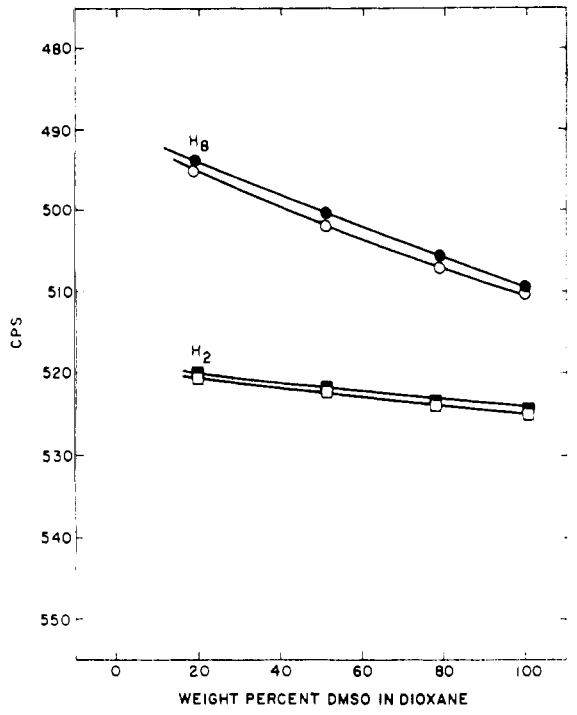


FIGURE 5: Proton chemical shifs of 6-methylpurine (at 32° relative to internal tetramethylsilane) *vs.* the weight per cent composition of DMSO–dioxane solutions. 6-Methylpurine concentration: 0.2 M (open symbols) and 0.04 M (solid symbols).

at highest field in the two solvents with the strongest basic properties, triethylamine and methanolic NaOH.

Discussion

The chemical shift data confirm that purine and its derivatives can undergo solute–solute and solute–solvent interactions which are strongly related to the nature and composition of the solvent medium. A qualitative description of the interactions can be obtained from the observed shift trends with solvent medium.

Nonaqueous Solvents and Mixtures. SOLUTE–SOLUTE INTERACTION. A wide variety of interactions is possible for purine compounds in nonaqueous media. Among the more important are (i) hydrogen bonding between appropriate proton donor and acceptor groups, (ii) dipole–dipole association, (iii), π-complex formation (analogous to the complexes formed between other polar aromatic molecules), and (iv) vertical stacking of the purine rings. Of these the latter three interactions would lead to significant upfield shifts of the ring proton signals with increasing solute concentration. Furthermore, both dipole–dipole and π-complex formation are strongly influenced by the dielectric properties of the solvent and a correlation might be expected between the ring proton shifts and the dielectric constant of the medium. From the data illustrated in Figures 4 and 5 and summarized in Table I it is clear that there is no significant concentration dependence of the ring proton shifts in any of the nonaqueous solvents and their mixtures, nor is there any correlation between the chemical shifts and the dielectric constants of these solvents. It is therefore unlikely that interactions ii–iv contribute significantly to shift changes of the H-8 protons. The absence of any base-stacking interaction in nonaqueous solvents confirms earlier work (Chan *et al.*, 1964; Katz and Penman, 1966).

TABLE I: Chemical Shifts of Purine[a] Protons in Organic Solvents.

Solvent	Dielectric Constant[b]	Chemical Shift[c]		
		H-6	H-2	H-8
1,4-Dioxane	2.21	545.0	531.9	491.1
$CDCl_3$	4.76	554.0	543.3	496.6
Acetonitrile	37.05	543.5	534.4	497.0
Nitromethane	35.0	543.6	534.0	501.3
Acetone	19.8	543.9	533.4	508.0
Methanol	31.8	545.4	536.1	512.5
DMSO	46	547.9	535.6	516.4
DMF	38.3	548.5	536.3	520.5
Triethylamine[d]	2.42	539.1	528.0	485.1
Methanolic NaOH[d]		527.5	516.8	492.1

[a] Concentration, <0.05 M. [b] Table of Dielectric Constants (1951), Department of Commerce, National Bureau of Standards, Washington, D. C. [c] Relative to internal tetramethylsilane. [d] Shifts reflect anion formation.

The self-association of purine and pyrimidine derivatives to form hydrogen-bonded dimers in chloroform and carbon tetrachloride solutions has been demonstrated in recent infrared and Raman studies (Kyogoku *et al.*, 1966, 1967a,b). X-Ray diffraction (Watson *et al.*, 1965) and infrared studies (Novak and Lautie, 1967) have also shown that purine forms intermolecular hydrogen bonds in the solid state between the N-7 hydrogen and the N-9 nitrogen atoms. A similar hydrogen-bonding interaction is likely to occur in nonpolar nonaqueous solvents. Although this would lead to a concentration dependence of the N-7 (N-9) proton signal,[3] little effect would be expected upon the ring proton shifts unless they participated directly in the hydrogen-bonding interaction. The present results indicate that this is not the case in nonaqueous media.

SOLUTE–SOLVENT INTERACTIONS. Interactions of this type fall into two categories: (i) those which are nonspecific, as for example, dispersion and reaction field effects; and (ii) those which are of a more specific character, the most important possibilities being hydrogen-bonding and dipole–dipole interactions. Since the H-2 and H-6 shifts are almost constant in all of the nonaqueous solvent mixtures whereas the H-8 proton

[3] That is, a shift to low field with increasing purine concentration.

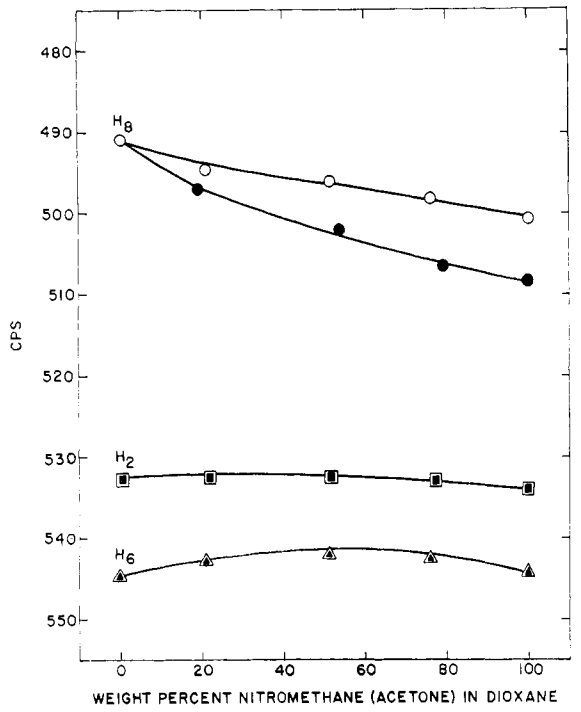


FIGURE 6: Proton chemical shifts of purine (0.02 M, at 32° relative to internal tetramethylsilane) *vs.* the weight per cent composition of dioxane–nitromethane (open symbols) and dioxane–acetone solutions (closed symbols).

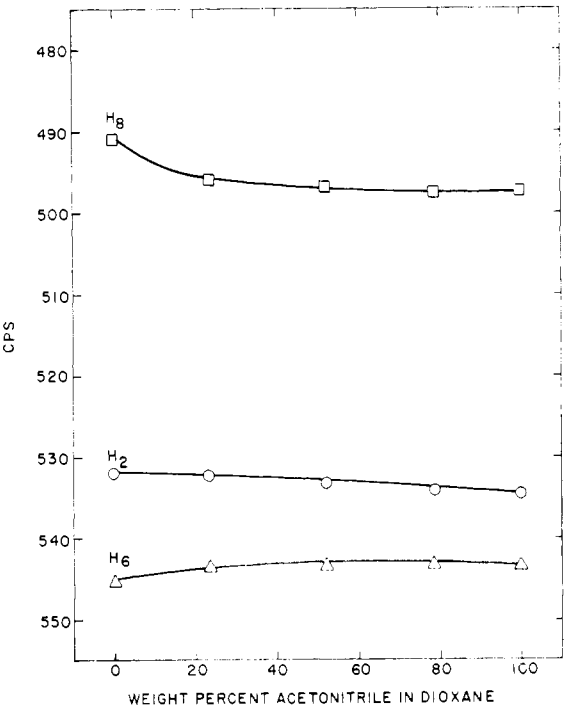


FIGURE 7: Proton chemical shifts of purine (0.013 M, at 32° relative to internal tetramethylsilane) *vs.* weight per cent composition of dioxane–acetonitrile solutions.

experiences marked shift changes, it is unlikely that dispersion effects predominate. It is also possible to rule out reaction field effects as the main cause of the shift changes for the H-8 proton. For example, if one considers a reasonable orientation of the electric dipole moment in purine, such that the positive end of the dipole is situated in the five-membered ring and the negative end is in the six-membered ring, then a simple calculation shows that an increase in the dielectric constant of the solvent should lead to a deshielding of the H-8 proton and a comparable shielding of the H-2 proton with little, if any, change predicted for the H-6 proton. None of these trends is observed for the purines and reaction field effects can have no more than a marginal influence upon the shifts. This is further confirmed by the observation of a significant temperature effect in dioxane–DMSO solutions. Such a temperature dependence would not be expected if nonspecific interactions were dominant.

An additional nonspecific shift change (predominantly affecting the H-8 proton) could also arise from the influence of solvent upon a possible tautomeric equilibrium of the imidazole N–H proton between the N-7 and N-9 positions. Although such an equilibrium would be solvent dependent and could conceivably have some effect upon the H-8 proton shift, this possibility is discounted because the shift changes for 9-ribosylpurine, in which a tautomeric equilibrium is not possible, are essentially the same as for purine and 6-methylpurine. In view of these considerations it is more reasonable to attribute the observed shift changes to a specific type of interaction.

From the relative constancy of the H-2 and H-6 proton shifts in different solvent mixtures it can be concluded that these protons are not located in regions directly affected by any specific solute–solvent interaction. Furthermore, the lack of any medium dependence for these protons would tend to preclude any significant formation of dipole–dipole or dipole–induced dipole (π complex) solvent–solute complexes. In each case the favored configuration of the complex is one in which the electron-deficient end of the solvent molecule is located over the electron-rich (pyrimidine) region of the purine (Schneider, 1962). This would lead to a noticeable solvent dependence of H-2 and H-6, which is not observed.

The deshieldings observed for the H-8 protons, on the other hand, are more in accord with a hydrogen-bonding interaction between this proton and suitable proton acceptor groups of the solvent molecules. Such an interaction is supported by several lines of evidence. It is well established that protons involved in hydrogen-bonding interactions are invariably shifted to low field with the magnitude of the shift change being roughly proportional to the acceptor properties of the solvent. A comparison of the H-8 shift data in Table I and Figures 4–7 indicates that the order of increasing shift change in different solvents (dioxane $\simeq$ acetonitrile < nitromethane < acetone < methanol < DMSO < DMF) roughly follows the proton acceptor properties of these molecules. A similar order has previously been observed for hydrogen-bonding shifts of $CHCl_3$ and other proton donor molecules (Howard *et al.*, 1963).

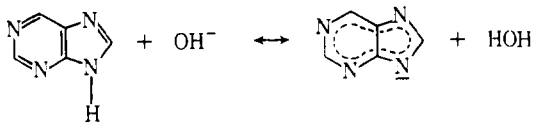
Further support for a hydrogen-bonding interaction is given by the results in binary solvent mixtures. In

each case addition of a stronger proton acceptor solvent of dioxane leads to a downfield shift of the H-8 signal. Again, the magnitude of the change increases with the proton acceptor strength of the added solvent. Although the largest initial slope of the solvent dependence curves is noted for the dioxane–D_2O system, as would be expected in view of the strong hydrogen-bond acceptor properties of water molecules, the over-all downfield shift trend is counteracted in this system by a stacking interaction between purine molecules.

The involvement of the H-8 proton in hydrogen-bonding interactions with solvent molecules is also indicated by the temperature measurements which show that the signal for this proton shifts significantly upfield in dioxane–DMSO solutions when the temperature is increased to 77°. In contrast, only a small change is noted for the H-2 and H-6 protons. Upfield shifts of hydrogen-bonded protons are commonly observed with increasing temperature and result from a decrease in the fraction of hydrogen-bonded species (Emsley *et al.*, 1965a).

Although the nuclear magnetic resonance measurements strongly indicate the involvement of H-8 protons of purines in hydrogen-bonding interactions with solvent molecules, they do not rule out the possibility of other hydrogen-bonding interactions. Of these, the most important would be interactions between N–H protons of the imidazole rings and acceptor centers of solvent molecules, and between nitrogen acceptors on the purines and suitable proton donor groups of the solvents. It is unlikely, however, that formation of a N–H---X hydrogen bond will have a significant deshielding effect[4] upon the H-8 shift since the shift trends for 9-ribosylpurine, where there is no possibility for such an interaction, are the same as for the other purines. In this connection it is interesting to note the similarity in solvent-dependent shift trends for the H-8 protons of purines and the α-proton on pyrrole and *N-n*-butylpyrrole (Schaefer and Schneider, 1960; Emsley *et al.*, 1965). As in the case of the purines low-field shifts were noted for the α-proton of pyrrole and its *N-n*-butyl derivative in the moderate proton acceptor solvent, acetone. Since hydrogen-bonding effects at the N position are precluded in *N-n*-butylpyrrole, it was concluded that a preferential hydrogen-bonding interaction takes place between the α-proton and the acetone and that any interaction at the N–H position in pyrrole does not have a dominant effect upon the shielding of the α-proton.

The purine ring proton shifts in methanolic NaOH and triethylamine solutions appear to be anomalous at first sight. In both cases the shifts are at much higher fields than would be expected from the strongly basic properties of the solvents. This is particularly evident when the shifts in methanol are compared with those in methanolic NaOH. A qualitative explanation of these upfield shifts can be given in terms of purine anion formation. Since the N-9 (N-7) proton of purines has acidic character (Katritzky, 1963), dissolution in strongly basic solvents would tend to favor the anionic form in these solvents, as represented by the equilibrium



In the anionic form the excess electron density is presumably redistributed *via* the π-electron system throughout the entire molecule and thereby leads to an increased shielding at each position on the ring.

Aqueous Solvent Mixtures. SOLUTE–SOLUTE AND SOLUTE–SOLVENT INTERACTIONS. The shift trends for the ring protons of purine derivatives differ very markedly in binary aqueous solutions from the trends in binary nonaqueous systems. Although addition of D_2O to dioxane initially leads to low-field shifts at concentrations below 60–70 wt % D_2O, further D_2O addition leads to upfield shifts. Furthermore, the chemical shift curves for all of the purine derivatives show a significant concentration dependence, which is most evident in D_2O-rich solutions. These results can be explained qualitatively in terms of a combination of solute–solvent and solute–solute interactions.

As in the case of nonaqueous proton acceptor solvents, the low-field shift of the H-8 proton can be attributed to a specific hydrogen-bonding interaction between this proton and water molecules. At D_2O concentrations below 10 wt % solute–solute interaction occurs to a limited extent only (as indicated by the small solute concentration dependence) and the H-8 shift is predominantly influenced by the hydrogen-bonding interaction. A comparison of the limiting slope ($D_2O \rightarrow 0$) for the H-8 chemical shift curve in aqueous mixtures with the corresponding slopes in nonaqueous mixture indicates that D_2O is a stronger proton acceptor than the strongest organic acceptor, DMF. This is in accord with the general order of proton acceptor strengths. The low-field shifts for the H-2 and H-6 protons with increasing D_2O concentration are somewhat larger than the changes noted in nonaqueous acceptor solvents. It is likely that these deshieldings are due to hydrogen-bonding interactions between D_2O and the ring nitrogens. However, in this case the interaction would be more favored at the adjacent N-1 atom which is known to have relatively strong acceptor properties (Cochrane, 1951).

The increasing dependence of the chemical shifts upon solute concentration in solvent mixtures with D_2O >10% and the upfield trends above 60% D_2O can be attributed to solute–solute interaction of the base-stacking type. Previous thermodynamic and nuclear magnetic resonance studies (Ts'o *et al.*, 1963; Ts'o and Chan, 1964) have shown that purine molecules are capable of aggregating in aqueous solution to form intermolecular stacks in which the purine rings are arranged in a face-to-face configuration. Such base-stacking interactions give rise to large upfield shifts of

[4] Based on the shift trends in methanolic NaOH and triethylamine solutions, it is more probable that formation of an N–H---X hydrogen bond would lead to an increased shielding at the H-8 position.

the ring proton signals with the largest differential shift changes occurring for the H-2 and H-6 (or methyl) protons. Based on the above it is reasonable to conclude that in the dioxane–D_2O mixtures addition of D_2O increases the fraction of stacked purines in solution. When the D_2O concentration is greater than 60% the shift contribution (upfield) from solvated (D_2O) purines in the stacked form becomes large enough to counteract the downfield shift due to hydrogen-bonding interactions.

Although chemical shift–concentration curves have been analyzed to obtain equilibrium constants for a variety of intermolecular interactions (Howard *et al.*, 1963), such a procedure is not feasible for the present systems because of their complexity. It can be noted, however, that the deshielding effect produced by hydrogen bonding with D_2O has a significantly greater influence upon the shift of the purine H-8 proton in aqueous solution, *i.e.*, −35 cps,[5] than the shielding contribution due to stacking, +15 cps. For the H-2 and H-6 protons, on the other hand, the upfield shifts due to stacking (~25 cps), are much larger than the contributions from hydrogen bonding (~12 cps).

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[5] The over-all hydrogen-bonding shift was taken as the difference between the shift in dioxane (where there is little if any solute–solute and solute–solvent interaction) and the extrapolated limiting shift in D_2O.