Interaction and Association of Bases and Nucleosides in Aqueous Solutions. VI. Properties of 7-Methylinosine as Related to the Nature of the Stacking Interaction

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Abstract: In a continuing study on the nature and the mechanism of association of nucleosides in aqueous solution, the properties of 7-methylinosine were investigated extensively. The results in pmr and conductance studies have supported the betaine-type structure of this molecule proposed previously. Therefore, this molecule is highly polar because of the deficiency of electrons in the five-membered ring and the excess of electrons in the six-membered ring. The vapor pressure osmometry data and the pmr results both indicate that this polar 7-methylinosine does not associate in water more than inosine or 1-methylinosine. This comparative study suggests that the electrostatic interaction of monopoles and dipoles of the bases does not contribute significantly to the stacking interaction of nucleosides in aqueous solution. A model for the stacking of 7-methylinosine is proposed based on pmr data. Concepts about the nature of the association of the nucleosides by stacking are proposed and discussed on the basis of current findings. Owing to the unusual structure of 7-methylinosine other properties of this molecule have also been investigated by pmr and uv absorption spectroscopy.

About 6 years ago, conclusive evidence was reported in the first paper of this series about the extensive association of bases and nucleosides in aqueous solution based on the studies of vapor pressure osmometry and solubility measurements.[2] As described in the subsequent papers in the series,[3-6] as well as in the contributions from other laboratories,[7-12] this important problem has been investigated further by other techniques such as proton magnetic resonances,[4-7,10] ultracentrifugation,[9,12] and calorimetry.[8,11] These studies show that the bases and the nucleosides associate extensively in aqueous solution by an exothermic process involving vertical stacking of bases. Stacking of nucleotides such as AMP has also been demonstrated recently by ultracentrifugation, pmr, and vapor phase osmometry (vpo) studies.[13,14] In the studies of cooperative binding of adenosine to polyuridylic acid, the stacking energy has been shown to be the major factor contributing to the conformational stability of nucleic acids.[15,16]

In the preceding paper of this series, the physical properties of 14 purine nucleosides in aqueous solution have been studied by vpo and pmr.[6] A model for the stacking has been proposed.[6,14] It was found that the tendencies of self-association among various nucleosides do not correlate with the dipole moment values of the corresponding bases. Since this conclusion is important in regard to the nature of molecular forces in the stacking interaction, it should be investigated further.

7-Methylinosine was first synthesized in one of our laboratories.[17] If the previously proposed betaine structure for this molecule is correct (Figure 1), then this molecule is highly polar because of the deficiency of electrons in the five-membered ring and excess of electrons in the six-membered ring. Thus, this molecule can be an ideal compound for evaluating the contribution of electrostatic interaction of monopoles and dipoles in the stacking interaction of nucleosides in aqueous solution. 7-Methylinosine was, therefore, investigated to ascertain its polar structure as well as its tendency to associate in water. Inosine and 1-methylinosine, which have been previously investigated by us,[6] serve as important controls for comparison throughout the study. The results confirm the proposed polar structure for 7-methylinosine in water and in DMSO, and indicate that the contribution of electrostatic interaction of monopoles and dipoles for the stacking interaction of nucleosides in water is insignificant. Owing to the unusual structure of 7-methylinosine, other interesting properties of this molecule have been investigated.

Experimental Section

7-Methylinosine was synthesized by the method described by Jones and Robins,[17] and, as reported, the compound softens at 137–139° and then decomposes. Results of several analyses re-

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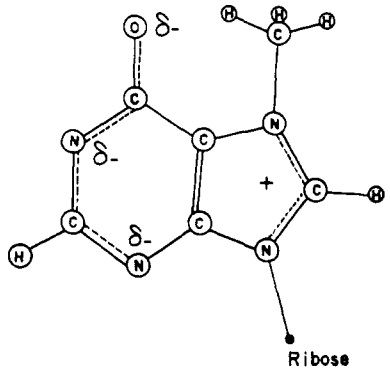

Figure 1. The polar structure of 7-methylinosine.

vealed that the compound is a hemihydrate (*Anal.* Calcd for $C_{11}H_{14}N_4O_5 \cdot 0.5\,H_2O$: C, 45.36; H, 5.19; N, 19.24. Found: C, 45.09 ± 0.5 (four determinations), H, 5.50 ± 0.2 (four determinations); N, 19.28). Elemental analysis was carried out by Mr. Joseph Walter, Johns Hopkins University, and by Spang Microanalytical Laboratory, Ann Arbor, Mich.

7-Methylinosine is unstable in aqueous solution (pH 8.2), though no detectable change of its uv spectrum in water occurs over 24 hr at room temperature. The pmr spectrum in D_2O also exhibits no detectable change for a period of 3 hr. After 3 hr a new peak gradually appears at 8.60 ppm, 10 cps upfield from that of the H-2 proton (8.7 ppm). The intensity of the H-2 proton of the 7-methylinosine decreases correspondingly to the increase of intensity of this new peak. After 24 hr, the intensities of these two peaks become equal. Although we have not yet identified this degradation product, the uv spectral data suggest that the ring system of the base is probably preserved. No such degradation process was detected when the compound was stored in the dry state. For this reason, all the pmr measurements and all the vpo measurements were made within 1 hr after the preparation of the aqueous sample. In buffered solution at pH 9.1, the decomposition occurs at a much faster rate. Twenty minutes after dissolving the compound in solution, it had decomposed to about the same extent as a pH 8.2 solution had after 3 hr. For this reason, the pmr studies on the 7-methylinosine in H_2O at pH 9.1 were done within 10 min after dissolving the compound.

The nucleoside solutions were all prepared in the two-component system containing only water (or D_2O) and the nucleoside of interest. The pH of the unbuffered 0.1 *M* 7-methylinosine solution was measured to be 8.2, of 0.1 *M* 1-methylinosine solution to be 7.85, and of 0.1 *M* inosine solution to be about 5.4. In one case, the 7-methylinosine was dissolved in 0.05 *M* Tris buffer at pH 9.1.

The pmr measurements were done with a Varian HA-100 spectrometer with the probe temperature at 28–30°. The values of the chemical shift are reported in parts per million from an external TMS capillary to ±0.005 ppm.

The vpo measurements were done at 25° with a Mechrolab 301A vapor pressure osmometer as described previously.[2,3] Even though all the measurements were completed in 1 hr after preparation of the aqueous sample, consistent readings were obtained within a period of 3 hr.

The conductance measurements were taken with a high precision Industrial Instrument, Inc. conductivity bridge at 0°. At 0.01 *M* and 0° conductance of 7-methylinosine solution was measured to be 15.5 ± 1.2 μmhos, a value larger than that of inosine solution (11 ± 1 μmhos). Ultraviolet spectra were taken with a Cary 14 spectrophotometer.

Results and Discussion

A. Structure of 7-Methylinosine in Water. It is of paramount importance in this investigation that 7-methylinosine in water indeed has a betaine structure as previously proposed.[17] Three lines of evidence from the pmr studies indicate the structure shown in Figure 1 is essentially correct for 7-methylinosine in water.

The first line of evidence comes from the comparison of the chemical shifts. In Table I, the chemical shifts of various protons of 7-methylinosine in D_2O and H_2O are compared with those of inosine and 1-methylinosine. Data at 0.1 *M* concentration and that extrapolated to infinite dilution are shown. The resonance of the methyl group of the 7-methylinosine is located about 0.6 ppm downfield from that of the 1-methylinosine. In addition, the concentration dependence of the chemical shift of the methyl protons of 7-methylinosine is much less (70% less) than that of the methyl protons of 1-methylinosine. The H-8 proton of 7-methylinosine could not be detected in D_2O even if the measurement was taken within 5 min after dissolving the compound in D_2O. This is undoubtedly due to the rapid exchange of the H-8 proton of 7-methylinosine with the deuterium in D_2O. In H_2O, however, the H-8 proton resonance was found to be a sharp line (2.5 cps line width) located about 1 ppm downfield from that of inosine and 1-methylinosine in D_2O (Table I, Figure 2). Also, the H-8 proton chemical shift of 7-methylinosine is practically not concentration dependent. Thus, the comparative pmr studies on 7-methylinosine, 1-methylinosine, and inosine in aqueous solution reveal that the chemical shifts of the H-8 proton and the methyl protons of the 7-methylinosine are located about 0.5–1.0 ppm downfield from those of the two other nucleosides and these values are not concentration dependent.

Table I. Proton Chemical Shifts of Inosine and Its Derivatives in Aqueous Solution (Parts per Million from TMS Capillary, 28–30°)

	Concn, *m*	H-8	H-2	H-1′	CH_3
A. D_2O					
Inosine[b]	0.0	8.75	8.65	6.52	
	0.1	8.68	8.56	6.44	
	a	0.07	0.09	0.08	0
1-Methylinosine[c]	0.0	8.72	8.77	6.49	4.09
	0.1	8.65	8.69	6.40	4.01
	a	0.07	0.08	0.09	0.08
7-Methylinosine	0.0		8.71	6.60	4.61
	0.1		8.60	6.51	4.58
	a		0.11	0.09	0.03
B. H_2O					
7-Methylinosine	0.0	9.68	8.72		
(pH 8.2)[d]	0.1	9.67	8.64		
	a	0.01	0.08		
	0.0	9.68	8.74	6.625	
(pH 9.1)[d]	0.1	9.67	8.65	6.565	
	a	0.01	0.09	0.06	

[a] These values of $\Delta\delta$ are in agreement (±0.01 ppm) with those published in ref 6 obtained from a 60-Mc instrument. [b] The δ values of inosine are the same as those published in ref 14 obtained from 100-Mc instrument but are 0.08–0.1 ppm (higher field) different from those published in ref 5 obtained from a 60-Mc instrument. [c] The δ values of 1-methylinosine are 0.08–0.1 ppm (higher field) different from those published in ref 6 obtained from a 60-Mc instrument. [d] The pH value of 0.1 *M* 7-methylinosine in water is measured to be 8.2. The pH 9.1 solution of 7-methylinosine was dissolved in 0.05 *M* Tris buffer.

The second line of evidence comes from the observations on the rapid exchange of the H-8 proton of 7-methylinosine just mentioned above. The H-8 proton of adenosine or inosine can be exchanged only to about 50% after heating at 80° in D_2O for 2 hr, while the exchange of the H-8 of 7-methylinosine is all completed within 3 min after dissolving in D_2O at room temperature. While the mechanism of the exchange of the H-8 of purine or adenosine is not yet extensively investigated, the unusually rapid exchange of the H-8 of the 7-methylinosine is explainable only on the basis of a positive

charge located nearby in the five-membered ring which labilizes the C_8-H linkage. These two lines of evidence indicate the presence of a positive charge in the five-membered ring (Figure 1).

Finally, the NH proton of inosine (0.064 *M*) in DMSO-d_6 was located at 12.68 ppm; while no NH proton can be found from solution of 7-methylinosine (0.092 *M*) in DMSO-d_6. After introduction of HCl (gaseous) into the solution of 7-methylinosine (0.23 *M*) in DMSO-d_6 the NH proton can now be found at a very low field position, 14.02 ppm from TMS. These data provide some valuable information about the structure of the six-membered ring. It indicates that the 7-methylinosine crystals as prepared do not contain an NH proton in contrast to the inosine which contains an NH proton in its six-membered ring. In addition, after protonation, an NH proton in 7-methylinosine can now be found. These data, therefore, also support the structure of 7-methylinosine as shown in Figure 1.

It is also of great importance that the degree of ionization of 7-methylinosine be known in aqueous solution. For this reason the pK_a of this compound was determined spectrophotometrically and was found to be 6.1 ± 0.1. The pH of the 0.1 *M* solution of 7-methylinosine was measured to be 8.2 (the original pH of the distilled water was about 6.4). At pH 8.2, the degree of protonation of 7-methylinosine is slightly less than 1%. This conclusion is supported by the pmr data listed in Table I. The δ values of the H-8 and H-2 protons of 7-methylinosine in phosphate buffer pH 9.1 are the same as those dissolved in distilled water which was measured to have a pH value of 8.2, indicating that at pH 8.2, 7-methylinosine exists essentially as a neutral molecule. In H_2O and at pH 4.9 (adjusted by adding HCl), the protons of 7-methylinosine (0.05 *M*) are located substantially downfield as anticipated from protonation; at pH 4.9, the δ value of H-8, H-2, and H-1′, respectively, are 9.89, 8.82, and 6.69 ppm.

B. Association and Stacking of 7-Methylinosine in Water. The molal osmotic coefficients of 7-methylinosine are given in Table II. Within the range of experimental errors, these three compounds appear to associate to about the same extent. The data suggest, however, that 1-methylinosine probably associates slightly more than the other two compounds. It should be mentioned that the values reported for 7-methylinosine are slightly too high because the 1% protonation was not taken into account in the calculations. This 2% error caused by protonation is within the range of our experimental variation and should not alter any of the conclusions. The inosine and 1-methylinosine are un-ionized when dissolved in neutral water solution.

Table II. Molal Osmotic Coefficients (φ) for the Following Compounds in Water at 25°

	Molal concn		
	0.05	0.10	0.15
Inosine[a]	0.957 ± 0.02	0.888 ± 0.01	0.830 ± 0.02
7-Methylinosine[b]	0.975 ± 0.02	0.875 ± 0.02	0.850 ± 0.02
1-Methylinosine[a]	0.926 ± 0.005	0.860 ± 0.005	0.800 ± 0.01

[a] Values obtained from ref 6. [b] Measurements were made within 1 hr from dissolving the compound in water. See Experimental Section.

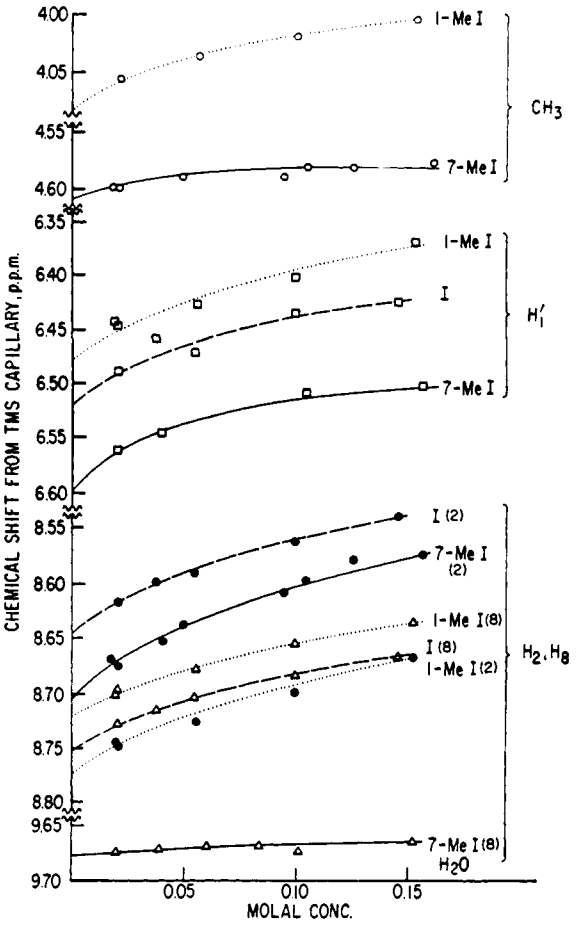

Figure 2. The concentration dependence of the chemical shifts of various protons of inosine, 1-methylinosine, and 7-methylinosine in D_2O at 28°; —○—○—, CH_3 proton; —□—□—, H-1′ proton; —●—●—, H-2 proton; —△—△—, H-8 proton.

The magnitude of concentration dependence of chemical shifts is represented by the value of Δδ shown in Table I. For the H-1′ proton, inosine, 1-methylinosine, and 7-methylinosine all have the same value of Δδ. For the H-2 proton, the Δδ for 7-methylinosine appears to be slightly larger (10–20%); this may be caused by an increase of aromaticity and, thus, an increase in the ring current in the six-membered ring of the 7-methylinosine due to resonance (Figure 1), in comparison to that of the other two compounds. As for the H-8 proton and the methyl protons, the Δδ for 7-methylinosine is practically nil, indicating no dependence on concentration of these two groups of protons in 7-methylinosine. The concentration dependence of all the pertinent protons of these three compounds are displayed in greater detail in Figure 2. Concentration dependence of 7-methylinosine was also studied in pH 9.1 buffer where the degree of protonation would be only 0.1% (Table I). This experiment gave results identical with those carried out in water; thus this 1% protonation of the 7-methylinosine in water does not interfere with the pmr interpretations.

Although 7-methylinosine possesses both positive (in the five-membered ring) and negative charges (in the six-membered ring) in the molecule (Figure 1), vapor pressure osmometry data and pmr measurement both indicate conclusively that this highly polar molecule does not self-associate or stack to an extent greater than

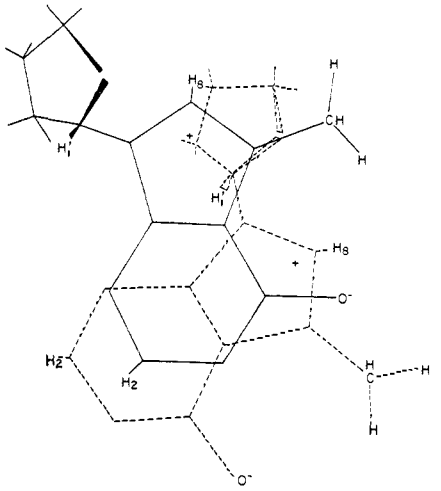

Figure 3. The proposed model for the stacking of 7-methylinosine in water.

that of inosine and 1-methylinosine, the neutral parent molecule or a closely related molecule of considerably less polarity. This conclusion will be discussed further in a later section.

The detailed information about the concentration dependence of the chemical shifts of H-2, H-8, H-1′, and CH_3 shown in Figure 2 provides a basis for constructing a model qualitatively describing the average orientation of two adjacent molecules in the stacks of 7-methylinosine. The proposed average orientation of two nucleosides in the stack is illustrated in Figure 3. In this proposed model, the H-2 proton of the upper molecule is shielded by the six-membered ring of the lower molecule while the H-1′ proton of the lower molecule is shielded by the five-membered ring of the upper molecule. The H-8 protons and the methyl protons are not shielded at all. Therefore, this model does satisfy the requirement of the pmr data in regard to the values of $\Delta\delta$ of different protons, *i.e.*, $\Delta\delta$ of H-2 is slightly larger than that of H-1′ while the $\Delta\delta$ of H-8 and $\Delta\delta$ of CH_3 are nil. This model, basically, is very similar to that proposed in our previous papers for stacking of nucleosides and nucleotides.[6] The six-membered ring is still on top of the six-membered ring, except now the orientation of the upper molecule is such that the center of its negative charge (N-1, C-6, and 6-oxo group) is on top of the center of the positive charge (C-8) of the lower molecule, and thus is away from the center of the negative charge of the lower molecule. Owing to charge repulsion, the five-membered ring, however, is no longer on top of the five-membered ring as in the model for the noncharged nucleosides (adenosine, inosine, etc.). Thus, the five-membered ring of the upper molecule is shifted over on top of the region of the H-1′ proton. For the consideration of electrostatic interaction, it is tempting to propose a model in which the six-membered ring of the upper molecule is over the five-membered ring of the lower molecule and the five-membered ring of the top molecule is over the six-membered ring of the lower molecule so that the positive regions and the negative regions of the upper and lower molecules overlap each other correspondingly and maximally. This arrangement, however, will not be able to bring about the shielding of the H-2 of either upper or lower molecules and, thus, is inconsistent with the present pmr data.

C. Properties of 7-Methylinosine and Related Compounds in Organic Solvents. Owing to the unusual structure of 7-methylinosine (Figure 1), its properties in organic solvents in comparison to those in aqueous solution have been investigated. Comparative studies were also made on inosine, 1-methylinosine, and adenosine.

1. Pmr Studies. In Table III the chemical shifts of H-8, H-2, H-1′, and CH_3 protons of inosine, 1-methylinosine, 7-methylinosine, and adenosine in both D_2O and DMSO are reported. All these values are referenced externally to a TMS capillary. With this external reference system, the changes of chemical shift of the methyl proton of methanol (0.4 *M*) and of acetone (0.4 *M*) from D_2O to DMSO are, respectively, 0.25 and 0.22 ppm upfield. The origin of this solvent upfield shift is probably the change of bulk magnetic susceptibility and the change of weak solute–solvent interaction.

For the purpose of discussion here, a solvent upfield shift of 0.2–0.3 ppm for protons being transferred from D_2O to DMSO will be considered as "normal." Into this "normal" category, we shall place the H-1′ protons of all these four compounds, the CH_3 protons of both 1-methylinosine and 7-methylinosine, and the H-2 protons of both inosine and adenosine. From this point of view, the solvent upfield shifts of the H-8 protons of all four compounds (0.09–0.04 ppm) are "abnormally" small, suggesting a bigger change of solute–solvent interaction in the opposite direction. The H-8 proton of purine nucleosides is known to be more "acidic" than other protons.[6,14,18,19] Hydrogen bonding of the H-8 proton of adenosine to DMSO has been previously suggested based on pmr results,[20] and recently this preposition was evoked again to explain the pmr results observed for the H-8 of purine in mixed solvent systems.[21] Crystallographic studies on the adenosine 3′-phosphate dihydrate indicated the existence of a C_8–H · · · O (water) hydrogen bond with a distance of H_8 to O (water) of 2.24 Å or C_8 to O (water) of 3.262 Å.[22] It appears, therefore, that the "acidic" H-8 proton is strongly hydrogen bonded to the receptors in DMSO solvent and is shifted to lower field. This is likely the reason why the solvent upfield shift of H-8 is small. The H-8 proton of 7-methylinosine is undoubtedly more "acidic" than those of the other three nucleosides and, interestingly, it has the smallest solvent upfield shift (Table III). In other words, the protons which do not interact strongly with DMSO, such as the methyl protons, H-1′ protons, etc., exhibit an 0.2–0.3-ppm solvent upfield shift when transferred from D_2O to DMSO. The "acidic" proton, such as that of H-8, which is likely to be strongly hydrogen bonded to the DMSO, exhibits only a 0.05–0.1-ppm solvent shift.

In this category of small DMSO-induced shifts, we have also found the H-2 proton of 1-methylinosine (Table III). The transfer of the 1-methylinosine from D_2O to DMSO induces an upfield shift of only 0.03 ppm for the H-2 proton, a value much less than those induced for the H-2 of other compounds (0.2–0.3 ppm). In our

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Table III. Comparison of Proton Chemical Shifts of Inosine, 1-Methylinosine, 7-Methylinosine, and Adenosine in D_2O and in DMSO (parts per Million from TMS Capillary, 28–30°)[a]

Compd	Solvent	Concn, *m*	H-8	H-2	H-1′	$-CH_3$
Inosine	D_2O	0[b]	8.75	8.65	6.52	
	DMSO	0.056[c]	8.66	8.40	6.20	
			0.09	0.25	0.32	
1-Methylinosine	D_2O	0[b]	8.72	8.77	6.48	4.05
	DMSO	0.043[c]	8.67	8.74	6.18	3.84
			0.05	0.03	0.30	0.21
7-Methylinosine	D_2O	0[b]	9.68 (H_2O)	8.72	6.51	4.60
	DMSO	0.062	9.64	8.35	6.24	4.30
			0.04	0.37	0.27	0.30
Adenosine	D_2O	0[b]	8.75	8.67	6.50	
	DMSO	0.05	8.69	8.48	6.22	
			0.06	0.19	0.28	

[a] The change of chemical shift of the methyl proton of methanol (0.4 *M*) and of acetone (0.4 *M*) from D_2O to DMSO (upfield) are, respectively, 0.25 and 0.22 ppm. [b] Extrapolated value to infinite dilution; accuracy is ±0.01 ppm. [c] Values of chemical shifts in DMSO are not concentration dependent.

Table IV. Ultraviolet Absorption Spectral Positions of Inosine, 1-Methylinosine, and 7-Methylinosine in Various Solvents

Solvent	Inosine			1-Methylinosine			7-Methylinosine[a]	
	λ_{max}	λ_{sh}[b]	λ_{min}	λ_{max}	λ_{sh}[b]	λ_{min}	λ_{max}	λ_{min}
Water	248		222	250		225	266	229
Methanol	249, 245	270	223	251, 246	267	225	273	233
Isopropyl alcohol	251, 245	265	225	252, 246	268	228	278	234
Acetonitrile	250, 243	266	233	252, 244	270	226	285	235
Dimethylsulfoxide	*c*	270	*c*	*c*	270	*c*	286	*c*

[a] The half-width is approximately the same in all solvents. [b] The intensity of the shoulder was approximately one-half the intensity of the major peak. [c] Masked by the absorption of the solvent.

previous publication,[6] we have already noticed that while the chemical shifts of H-8 protons of inosine and 1-methylinosine are almost the same, the chemical shifts of H-2 protons of 1-methylinosine is downfield by 0.12 ppm as compared to that of inosine. As a matter of fact, among all the 6-substituted purine nucleosides known, 1-methylinosine is the only one which has the δ of H-2 at a lower field than δ of H-8.[6] Our interpretation was that there is substantial quaternization occurring at N-1 in the 1-methyl derivative as compared with inosine itself, which provides the substantial and selective deshielding of H-2 and 1-methylinosine. This same process also increases the "acidity" of the H-2 proton in 1-methylinosine, just as the quaternization of N-7 in 7-methylinosine increases the "acidity" of the H-8 proton. Therefore, we tentatively conclude that the small DMSO-induced shift of H-2 of 1-methylinosine is again related to its relatively higher "acidity," as in the case of the H-8 proton.

On the other hand, the DMSO-induced shift for the H-2 proton of 7-methylinosine is "abnormally" high, 0.37 ppm (Table III). The proposed rationale for this observation is the following. In water, the negative charge tends to locate at the 6-oxo group outside the six-membered ring (Figure 1). In this form of negative charge distribution, the negative-charge density of the six-membered ring tends to decrease, and the aromaticity, thus the ring current, tend to increase. Both these consequences lead to a relatively large downfield shift of the H-2 proton of 7-methylinosine in water, *i.e.*, 8.72 ppm as compared to 8.65 ppm of inosine. In DMSO, on the other hand, the negative charge tends to migrate partially back to the ring which will bring about higher negative-charge density and less aromaticity. Both events lead to a relatively large upfield shift of the H-2 proton of 7-methylinosine in DMSO, *i.e.*, 8.35 ppm as compared to 8.40 ppm of inosine. Thus, the difference between the H-2 proton shift in D_2O and that in DMSO becomes "abnormally" large. This phenomenon also supports the betaine structure for the 7-methylinosine (Figure 1) concluded in section A. Addition of small amounts of alkali to the DMSO solution of 7-methylinosine caused no change in the spectrum. This experiment ensures that the spectrum of 7-methylinosine in DMSO is from the unprotonated species.

2. Ultraviolet Absorption Spectroscopy. The uv spectra of inosine and 1-methylinosine in organic solvents are more complex than those in water (Table IV). In organic solvents, a prominent shoulder is developed in the 265–270-mμ region and the maximum peak is split with little change in the λ_{max} position. The probable explanation for the appearance of the shoulder is a bathochromic shift of the $n \rightarrow \pi^*$ transition, previously buried under the $\pi \rightarrow \pi^*$ transition region in water, when the nucleosides are placed in solvents of less hydrogen-bonding capacity. This notion is supported by the observation that the spectrum of 9-methylhypoxanthine has a prominent shoulder in the vapor phase also.[23] In the vapor phase, the λ_{max} of the spectra of 9-methylhypoxanthine exhibits a 10-mμ hypsochromic shift as well, which may be related to the appearance of the 245-mμ peak of the maximum region in the spectra obtained in organic solvents. Clark, *et al.*,[23] attribute this shift of the transition as "a greater London attraction of the solvent for the excited state than the ground state of the molecule."

For 7-methylinosine, no shoulder is formed for its uv spectra in organic solvents. Instead, a very significant

(23) L. B. Clark, G. P. Peschel, and I. Tinoco, Jr., *J. Phys. Chem.*, **69**, 3615 (1965).

bathochromic shift of the spectral maximum is observed with a magnitude of 8–20 mμ (Table IV). This large shift of λ_{max}, however, does not bring about much change in extinction coefficient; the λ_{max} for 7-methylinosine was reported to be 8.4×10^3 in water,[17] and was found to be 8.2×10^3 in DMSO. Addition of water to the DMSO solution up to 10 mole % (or about 2.5 vol %) brought about a 1-mμ hypsochromic shift of the λ_{max}, and addition of 20 mole % of water brought about 3-mμ hypsochromic shift. At present, the nature of this shift of λ_{max} of the spectra in water as compared to that in organic solvent is not well understood. The most plausible interpretation is that, since the ground state of the 7-methylinosine is so highly polar already, the excited state for the transition may become less polar.[24] If so, then the transition energy between the polar ground state and the less polar excited state will be increased when the compound is transferred from an organic solvent to water, because the polar ground state will be stabilized preferentially in water over the excited state. Thus, the λ_{max} of the spectrum in water will be at a shorter wavelength as compared to that in organic solvents as observed.

D. Nature of the Stacking Association. Since the original finding about the extensive association of bases and nucleosides in water,[2] three main concepts have been developed in describing the nature of this interaction. First, the mode of association is by vertical stacking of the bases and not by horizontal hydrogen bonding.[2-7] The degree of association certainly proceeds much beyond the dimer stage.[2-4,6,8,9,11,12] Second, the association is exothermic.[2,4,8-11] The enthalpy for the association is about −2 to −4 kcal and the over-all entropic contribution is always negative, ranging from −5 to −16 eu.[8,9,11] Third, the extent of association of the purine derivatives is greater than that of the pyrimidine derivatives.[2,3,6,8,11,12] Methylation at the base moiety usually enhances the association while methylation at the pentose moiety has little effect.[6] The range of the free energy for the association at 25° is from +300 cal (uridine) to −1.9 kcal (N-6 dimethyl adenosine).[6,8,9,11,12] At present, the experiments described above strongly indicate that electrostatic interaction of permanent monopoles and dipoles do not provide the free energy of association in water. Though the contribution of electrostatic interaction derived from theoretical considerations is very substantial in the vacuum state ($F_{\rho\rho}$, $F_{\rho\mu}$, $F_{\mu\mu}$),[25] the present experimental conclusion is perhaps not too surprising. If electrostatic interaction of fixed charges is of primary importance, and if the solute–solute interaction is not dominantly influenced by the strong solvent–solvent interaction, then the self-stacking of the bases in organic solvent of low dielectric constant should be much greater than that in water, which it is not. In fact, bases do not self-associate in organic solvent by stacking but form hydrogen-bonds with the specific, complementary bases.[20,26-32] Also, positive and negative ions such as Na^+ and Cl^- do not associate to a great extent in water in the range of 0.01–0.1 *m* concentration. It should be noted that it is entirely possible that the charged properties of 7-methylinosine have made a contribution to the negative enthalpy for the association. This contribution, however, is counterbalanced by the negative entropy with no resultant increase in the negative free energy for the association. This important possibility hopefully will soon be investigated.

Though the nature of the molecular force(s) in providing the negative enthalpy for the association is not well understood at present, these forces undoubtedly are the cause for the mode of association stacking of the planar bases. Some indications do exist that there is an entropic contribution to the association though the over-all entropic value is negative, opposing the association. For instance, the free energy of association for caffeine (−1.5 kcal) is definitely larger than that of purine (−0.44 kcal) or 6-methylpurine (−1.12 kcal), yet the enthalpy for purine (−4.2 kcal) or 6-methylpurine (−6.0 kcal) is substantially larger than that of the caffeine (−3.4 kcal).[2,3,6,8] The reason for a larger negative free energy for the self-association of caffeine comes from a smaller negative entropy (−6 eu) *vs.* that of purine (−13 eu) or that of 6-methylpurine (−16 eu).[8] Similarly, it appears that the larger negative free energy for the self-association of purine riboside (−0.4 kcal) over that for the pyrimidine nucleosides (+0.08 to +0.3 kcal) comes from the smaller negative entropy (−7 eu) for the purine nucleosides *vs.* that of the pyrimidine nucleosides (−10 eu), since the enthalpy for purine riboside association (−2.5 kcal) is actually smaller than that of the pyrimidine nucleosides (about −2.75 kcal).[11] These two comparisons strongly suggest the existence of a positive entropic contribution to the association, hidden in the over-all negative entropic value. Such a positive entropic contribution most likely is derived from the competition between solute–solvent (water) interaction and solvent–solvent (water) interaction. Very recently, Crothers and Ratner[33] have described a thermodynamic study on the association system of actinomycin with deoxyguanosine which may be relevant for the discussion here. The association process of actinomycin with deoxyguanosine has a negative enthalpy and a negative entropy, which is similar to the situation of nucleoside association. A hidden, *positive* entropic contribution in the aqueous system can be demonstrated, since the entropy value becomes *more negative* upon increase of the methanol content of the system. They concluded that this is a demonstration of the "hydrophobic bonding" through the ordering water molecules around the dissolved particles.[33]

This notion of the hidden positive entropy contribution in the aqueous system and the fact that the self-stacking of bases does not take place in organic solvents but only in water indicate that the early description of this interaction as "hydrophobic-stacking interaction"[2] may be rather appropriate. As a working hypothesis, we view that the bases are clustered in

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aqueous solution because of the strong self-interaction of water molecules. With the bases in a close proximity to each other, short-ranged, attractive forces (forces proportional to $(1/r)6$ or even to $(1/r)3$) begin to become dominant, which most likely involve π electrons polarizabilities and energies through electrostatic interaction.[6,34]

The recent paper by Helmkamp and Kondo[35] is of significance in providing further understanding about the nature of the molecular forces participating in the association. They reported that the apparent free energy for self-association (ΔF, cal/mole) of 9-methylpurine in aqueous solution at 25° is −380, while the ΔF for purine and 6-methylpurine are −440 and −1100, respectively.[2,3] The concentration dependence of the chemical shifts ($\Delta\delta$) of the protons of 6-methylpurine[4] correspondingly is much higher than those of 9-methylpurine and purine.[4,35] This difference between the C-6 methylation *vs.* N-methylation is unlikely to be due to the loss of contribution of hydrogen bonding to the association, by the removal of the N-H in the N-9 methylation. The reasons are that these molecules associate mainly through stacking, as shown by the upfield shift observed,[2,3,35] and that they do not associate in organic solvents. At present, there is also no obvious reason why this difference is caused by a steric effect. The best explanation we can propose is that the N-9 alkylation has blocked the tautomerization of the N-H between the N-9 and N-7 at the five-membered ring of the purine derivatives. The blockage of this tautomerization causes a reduction in the allowable patterns of electron distribution in the system and should, therefore, decrease the polarizability of the molecule. This decrease may lead to a lowering of the tendency of association in stacking. Comparison of the thermodynamic qualities of the association with the polarizability of these compounds will be of significant value. Helmkamp and Kondo[30] have also reported that the substitution of isopropyl or *t*-butyl at the N-9 position will have a much smaller effect on the extent of association of the alkylated compound than when substitution by these groups is at C-8, C-2, or C-6 positions. However, the concentration dependence of the chemical shifts ($\Delta\delta$) observed for the N-9 alkylated compounds is slightly higher than for those substituted at various carbon atoms. These data imply the possibility that the N-9-substituted compounds may have a different mode of association than that of the carbon-substituted compounds, besides having comparatively lower association constants.

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Communications to the Editor

Organometallic Pnicogen Complexes.[1] III. Preparation and Structural Characterization of the Triarsenic–Cobalt Atom Cluster System $As_3Co(CO)_3$. The First Known X_3–Transition Metal Analog of Group Va Tetrahedral X_4 Molecules[2,3]

Sir:

A comprehensive investigation of the field of arsenic–metal carbonyl clusters has resulted in the isolation and characterization of a truly unusual molecule, $As_3Co(CO)_3$, which represents another member of the arsenic–metal cluster series derived by the successive replacement of As atoms in the tetrahedral As_4 molecule with electronically equivalent $Co(CO)_3$ groups.[3,4] The synthesis and X-ray investigation of this remarkable arsenic–metal carbonyl complex, which is distinguished from yellow As_4 (and white P_4) by high air stability and striking ease of formation,[5,6] was a consequence of our attempt to prepare a group Va analog of the antiaromatic chalcogen metal atom clusters $XCo_3(CO)_9$ (X = S, Se).[7-9]

The reaction of $Co_2(CO)_8$ and $[AsCH_3]_5$ in hexane at 200° under 100 atm of carbon monoxide yields a variety of products. Chromatography on silica gel with hexane elutes pure $As_3Co(CO)_3$, and sublimation in a sealed tube at 35° and 10 mm pressure produces clear yellow hexagonal plates. The mass spectrum of this diamagnetic complex[10] shows not only all As_3Co-

(1) The terms "pnicogen" (Greek, πνιγμός—a choking, suffocation) and "pnictide" have been introduced as group names for the group Va family of elements (N, P, As, Sb, and Bi) analogous to the use of "chalcogen" and "chalcogenide" as group names for the group VIa elements. The fact that these terms have been gaining scientific favor in the past several years (as stated by E. F. Westrum, Jr., *Progr. Sci. Technol. Rare Earths*, **2**, 76 (1966) is typified by their usage in the following references: F. J. Kohl, J. E. Prusaczyk, and K. D. Carlson, *J. Am. Chem. Soc.*, **89**, 5501 (1967); A. T. Howe and P. J. Fensham, *Quart. Rev.* (London), **21**, 521 (1967).
(2) For previous papers in this series, see (I) J. M. Coleman and L. F. Dahl, *J. Am. Chem. Soc.*, **89**, 542 (1967); (II) L. F. Dahl, W. R. Costello, and R. B. King, *ibid.*, **90**, 5422 (1968).
(3) Presented in part at the 157th National Meeting of the American Chemical Society, Minneapolis, Minn. April 1969.
(4) A. S. Foust, M. S. Foster, and L. F. Dahl, *J. Am. Chem. Soc.*, **91**, 5633 (1969).
(5) The yellow arsenic allotrope, As_4 (which is less stable than white P_4), is metastable under all conditions. When exposed to light even at −180° it rapidly changes to gray arsenic (isomorphous with black phosphorus); it is rapidly oxidized by air at room temperature.[6]
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(9) C. E. Strouse and L. F. Dahl, submitted for publication.
(10) Faraday magnetic measurements at room temperature kindly performed by Mr. Michael Camp confirmed the expected diamagnetism.