retention time (3.15 min) on a 6-ft 5% DEGS column at 175°, by its R_f value on TNF-impregnated silica gel,[18] and by its nmr spectrum (four benzylic protons at τ 7.20), all identical with those of an authentic sample.

1,6- (or 3,6-) Dihydropyrene. The reduction of pyrene with lithium in ammonia–THF was quenched after 1 hr as rapidly as possible with water, and the product precipitated by addition of excess water. Tlc on silica gel in cyclohexane indicated the presence of only a single component (R_f 0.11) in addition to unreacted pyrene (R_f 0.28). Gas chromatography on a 6-ft 5% DEGS on Chromosorb W column at 175° similarly exhibited one product (14.6 min) and pyrene (24.2 min). The nmr spectrum of the mixture was consistent with the presence of the predicted 1,6- (or 3,6-) dihydropyrene structure (see Discussion). However, decomposition (primarily isomerization to 4,5-dihydropyrene) took place on both the gas chromatographic column and a column of silica gel at room temperature, preventing isolation of the 1,6- (or 3,6-) dihydro isomer. The identity of 4,5-dihydropyrene was demonstrated by correspondence of the nmr spectrum (notably, the presence of a singlet for four benzylic protons at τ 6.80) and retention time on the glpc column identical with that of the authentic compound.[41]

9,9,10-Triethyl-9,10-dihydroanthracene. To a solution of II ($R = C_2H_5$) (1.18 g, 5 mmol) in THF (50 ml) and ammonia (150 ml) was added a solution of ethyllithium in benzene (5 mmol). After 15 min the red color was discharged by the addition of excess ethyl bromide. Glpc analysis of the oil (1.06 g) obtained upon work-up indicated II ($R = C_2H_5$) (3%) and the 9,9,10-triethyl compound (97%). The assigned structure was confirmed by nmr spectroscopy: 10 (t, 3, CH_3, $J = 7$ Hz), 38 (t, 3, CH_3, $J = 7$ Hz), 46 (t, 3, CH_3, $J = 7$ Hz), 113 (m, 2, CH_2CH), 117 (q, 2, CH_2, $J = 7$ Hz), 124 (q, 2, CH_2, $J = 7$ Hz), 240 (t, 1, CH, $J = 6$ Hz), and 436 cps (m, 8, aryl).

Anal. Calcd for $C_{20}H_{24}$: C, 90.85; H, 9.15. Found: C, 91.28; H, 8.84.

Attempted ethylation of I ($R = C_2H_5$) under similar conditions provided the triethyl compound (18%) and unreacted starting material (82%).

Acknowledgment. This investigation was supported in part by U. S. Public Health Service Research Grant CA-08674 from the National Cancer Institute. Microanalyses were performed by Micro-Tech Laboratories, Inc., Skokie, Ill.

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[41] E. A. Coulson, *J. Chem. Soc.*, 1298 (1937).

NH–Proton Exchange of Purine in Aqueous Solution. Effect of Molecular Complexing on Reaction Rate[1]

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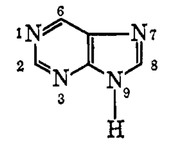
Contribution from the Lecks Chemical Laboratories, Brandeis University, Waltham, Massachusetts 02154. Received October 15, 1968

Abstract: The effect of molecular complex formation on the kinetics of NH–proton exchange of purine was studied by the nmr method in aqueous solution at pH 4.2–7.0. At low purine concentrations, the following fast reactions were characterized by kinetic analysis: (a) proton transfer with second-order kinetics involving the reactants purine, water, and purinate ion; rate constant $k_2 = 1.0 \times 10^8$ sec^{-1} M^{-1} at 20°; (b) reaction of purine with hydrogen ion: rate constant $k_{H^+} = 2.4 \times 10^{10}$ sec^{-1} M^{-1} at 20°. At 0.85 *M*, where the average degree of association of purine is 2.01, the rate constants are nearly the same: $k_2 = 1.0 \times 10^8$ and $k_{H^+} = 3.1 \times 10^{10}$ sec^{-1} M^{-1} at 20°. The high value of k_{H^+} implies that purinium ion can undergo very rapid proton exchange at N-9, perhaps by a mechanism of reversible ionization. The purine NH–water OH proton chemical shift was found to be 12.4 ppm at 20°.

We wish to report nmr measurements of the kinetics of proton exchange between purine (PuH) and water in the presence of 0.1 *N* NaCl. It has been shown by a variety of methods that purine molecules associate in aqueous solution to form molecular stacks,[2–5] and we were interested in the effect of that association on proton exchange. Our results may also be pertinent to current work on the mechanism of proton exchange between nucleic acids and water,[6] by providing data for a simple model system.



I

Our measurements were made in the pH range 4.2–7.0, in which purine exists largely in the form of the uncharged species, PuH. The measured proton exchange is between NH sites of PuH and OH sites of water. The purine concentration ranged from 0.036 to 0.85 F.

Osmotic data for purine and its derivatives are consistent with the model that association takes place to an infinite degree, and that the equilibrium constants for consecutive association steps are all equal.[2,5] Let [PuH] denote the formal purine concentration, let $[X_1]$ and $[X_k]$ denote molar concentrations of purine monomer and k-mer, and let K denote the stepwise association constant (eq 1), which by hypothesis is independent of k. For purine in water, $K = 2.4$ at 20° and $\Delta H°$

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[1] Work supported by the Petroleum Research Fund of the American Chemical Society. Grateful acknowledgment is made to the donors of that fund.

[2] P. O. P. Ts'o, I. S. Melvin, and A. C. Olsen, *J. Am. Chem. Soc.*, **85**, 1289 (1963).

[3] S. I. Chan, M. P. Schweizer, P. O. P. Ts'o, and G. K. Helmkamp, *ibid.*, **86**, 4182 (1964).

[4] S. J. Gill, M. Downing, and G. F. Sheats, *Biochemistry*, **6**, 272 (1967); E. L. Farquhar, M. Downing, and S. J. Gill, *ibid.*, **7**, 1224 (1968).

[5] G. K. Helmkamp and N. S. Kondo, *Biochim. Biophys. Acta*, **157**, 242 (1968); **145**, 27 (1967).

[6] See, for example, M. P. Printz and P. H. von Hippel, *Proc. Natl. Acad. Sci. U. S.*, **53**, 363 (1965); S. W. Englander and J. J. Englander, *ibid.*, **53**, 370 (1965).

$$K = [X_k]/[X_1][X_{k-1}] \qquad (1)$$

= −4.2 kcal.[2–4] Thus in our experiments at 20°, the average degree of association $\langle n \rangle$ (eq 2) varied from 1.08 at the lowest to 2.01 at the highest purine concentration.[7]

$$\langle n \rangle = \sum k[X_k]/\Sigma[X_k] = (1 - K[X_1])^{-1} \qquad (2)$$

Within the accuracy of our data, the rate of proton exchange, R, is reproduced by rate law 3. Most of the rate can be accounted for by the kinetic terms with coefficients k_{H^+} and k_2. The third, pH-independent, term with coefficient j_2 is quite insignificant at low purine concentrations and perhaps also at high purine concentrations, since it accounts at most for 10% of R when [PuH] is 0.85 F. If the term is taken to be real, then the pH-independent reaction is at least second, and perhaps higher, order in [PuH]. We therefore write $j_2[\mathrm{PuH}]^2$, using the symbol j_2 rather than the conventional k of rate constants to emphasize the uncertainties.

$$R = k_{H^+}[\mathrm{PuH}][\mathrm{H}^+] + k_2[\mathrm{PuH}][\mathrm{Pu}^-] + j_2[\mathrm{PuH}]^2 \qquad (3)$$

Results obtained at 20.4° are summarized in Table I. It is seen that k_2 is independent of [PuH] within the experimental precision, which is about 5%. The increase in k_{H^+} is significant but too small to warrant much discussion. We suggest that reaction involving PuH, water, and $\mathrm{PuH_2}^+$ is not quite negligible at the higher purine concentration. In either case, the data show clearly that molecular stacking of purine does not reduce the rate constant for proton transfer.

Table I. Proton Exchange Kinetics and Acid Dissociation of Purine in 0.1 N NaCl in Water at 20.4°

Parameter	[HPu], F 0.0366	0.8505
$\langle n \rangle^a$	1.080	2.005
k_{H^+}, sec^{-1} M^{-1}	2.4×10^{10}	3.1×10^{10}
k_2, sec^{-1} M^{-1}	0.95×10^8	1.01×10^8
j_2, sec^{-1} M^{-1}	$(1.6 \times 10^4)^b$	1.6×10^4
pK_{A1}	2.596^c	2.596^c
$\Delta H_{A1}°$	1.25 ± 0.29	1.25 ± 0.29
pK_{A2}	8.937^c	8.937^c
$\Delta H_{A2}°$	6.99 ± 0.14	6.99 ± 0.14

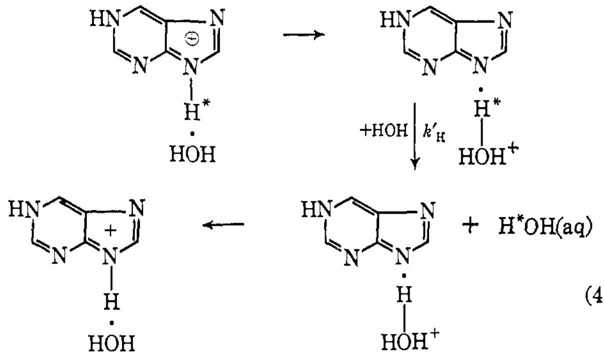
[a] Number-average degree of association (eq 2), K = 2.37 at 20°. [b] Use the same value as observed with 0.85 N purine. [c] Measured in 0.1 N NaCl, 0.01 N purine at 20, 30, 40, and 50°.

The results are noteworthy also for other reasons. The term, $k_2[\mathrm{PuH}][\mathrm{Pu}^-]$, in eq 3 measures the rate of a symmetrical proton-transfer reaction in which the reactants are PuH, water, and Pu^-. The number of water molecules that participate in this reaction cannot be deduced from our data, but Dr. Arthur Katz, in recent nmr measurements involving ^{17}O-labeled water, has found that number to be 1.1 ± 0.2.[8a] As far as we know, this is the first rate measurement for such a process in which the proton donor is an uncharged NH acid. The rate constant, k_2, is high—similar to that for the analogous termolecular reaction involving the uncharged OH acid, phenol.[8b]

The rate constant, k_{H^+}, for reaction of hydrogen ion with PuH is almost exactly equal to the rate constant, k_{-a}, for reaction of hydrogen ion with imidazole: in water at 20.4°, $k_{H^+} = 2.4 \times 10^{10}$ sec^{-1} M^{-1}, $k_{-a} = 2.1 \times 10^{10}$ sec^{-1} M^{-1}.[9] The latter reaction results in the formation of imidazolium ion, apparently at each encounter. Because of the close similarity of rate constants and substrate structures, it is reasonable to identify k_{H^+} with the rate constant for the reaction, $\mathrm{PuH + H_3O^+ \rightarrow PuH_2^+ + H_2O}$. Current views have it that $\mathrm{PuH_2}^+$ is an equilibrium mixture of tautomeric isomers, with one proton bonded largely at N-9 and the other distributed over N-1, N-3, and N-7 (see I).[10] The mean lifetime of $\mathrm{PuH_2}^+$ is probably too short to permit extensive scrambling of NH protons: the rate constant for acid dissociation, $\mathrm{PuH_2^+ + H_2O \rightarrow PuH + H_3O^+}$, is $2.4 \times 10^{10} \times K_{A1}$, or 6×10^7 sec^{-1} at 20°.

Next let us consider how the cycle, $\mathrm{PuH \rightarrow [PuH_2^+] \rightarrow \rightarrow PuH}$, in which $[\mathrm{PuH_2}^+]$ is a short-lived intermediate, can result in NH–OH proton exchange with the high observed rate constant. It is unlikely that the two NH protons in $\mathrm{PuH_2}^+$ become equivalent by scrambling. But even if they did, the probability of proton exchange per cycle would be only 0.5, and k_{H^+} would be only one-half the observed value. We must therefore conclude that $\mathrm{PuH_2}^+$ can undergo proton exchange efficiently without acid dissociation.

Proton exchange without acid dissociation has been demonstrated to be very fast for imidazolium ion in water and characterized as involving an ionized intermediate.[9] We propose a similar mechanism here. For example, the steps by which a proton might exchange at N-9 in one of the tautomeric isomers are shown in eq 4.



(4)

Experimental Section

Materials. Purine (from Mann Biochemicals Co.) was recrystallized twice by dissolving in hot methanol and letting needles grow overnight at −5° after reducing the volume of the solution by evaporation at reduced pressure. The crystals were washed with ether at −5° and dried *in vacuo*, mp 216–217° (to give a homogeneous yellow-brown liquid); neutralization equivalent with NaOH, within 0.5% of theory; spectral properties in H_2O: λ_{max} 263 mμ (ϵ_{max} 8740 M^{-1} cm^{-1}); in 0.1 N HCl: λ_{max} 260 mμ (ϵ_{max} 6950 M^{-1} cm^{-1}).

9-Methylpurine[5] was a gift from Professor G. K. Helmkamp; mp 160–161°; spectral properties in water: λ_{max} 263 mμ (ϵ_{max} 7400); λ_{max} 202 mμ (ϵ_{max} 16,100). Sodium chloride was a certified reagent. Water was pure laboratory reagent and was redistilled through an all-glass apparatus in the final kinetic measurements.

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(7) For theory of association processes that take place to an infinite degree, see E. N. Lassettre, *J. Am. Chem. Soc.*, **59**, 1383 (1937); J. A. Schellman, *Compt. Rend. Trav. Carlsbad, Ser. Chim.*, **29**, 223 (1956).

(8) (a) We are indebted to Dr. Arthur Katz for doing these measurements. The experimental method is that of Z. Luz and S. Meiboom, *J. Chem. Phys.*, **39**, 366 (1963). (b) E. Grunwald and M. S. Puar, *J. Phys. Chem.*, **71**, 1842 (1967).

(9) E. Grunwald and E. K. Ralph, *J. Am. Chem. Soc.*, **91**, 2422 (1969).

(10) J. M. Read and J. H. Goldstein, *ibid.*, **87**, 3440 (1965).

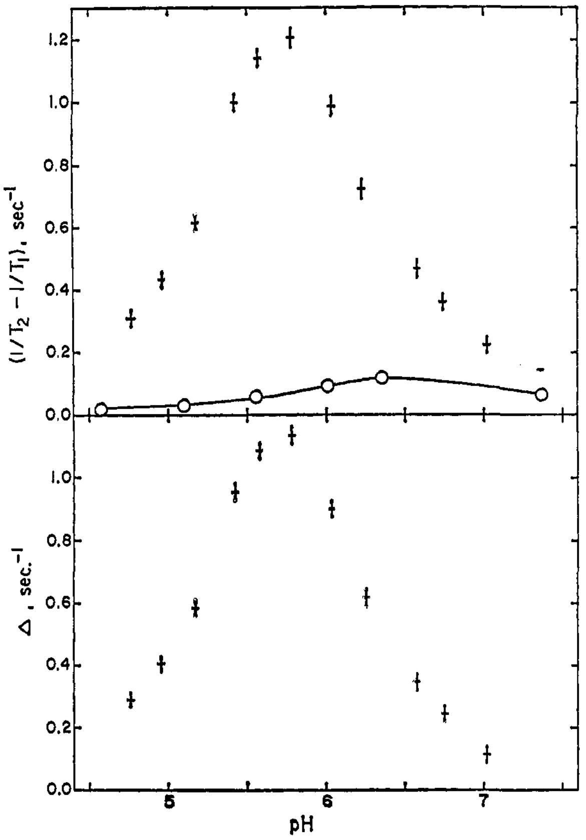

Figure 1. Evaluation of Δ as a function of pH for 0.85 *M* purine in 0.1 *N* aqueous NaCl at 20.4°. Upper figure, $(1/T_2) - (1/T_1)$ in the presence (crosses) and absence (circles) of purine. Lower figure, the difference, Δ, which measures the exchange broadening ascribable to OH-NH proton exchange.

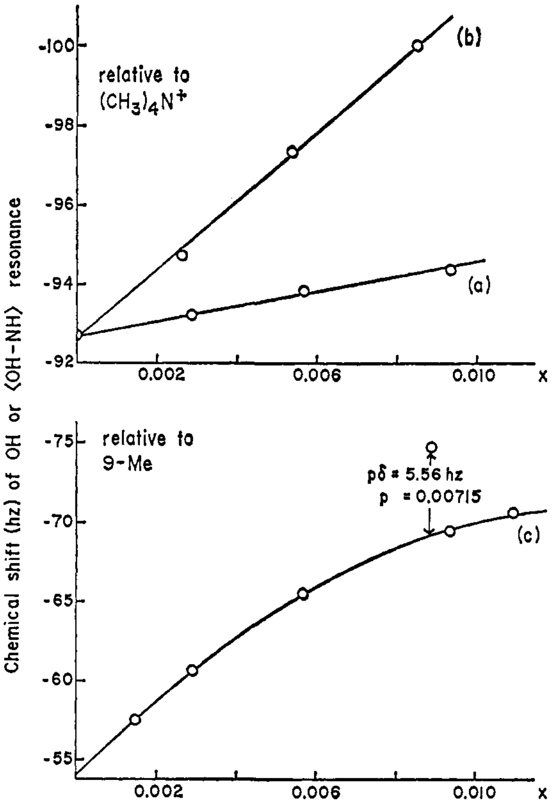

Figure 2. Chemical shift of the OH or <OH-NH> proton resonance in water at 20.4° as a function of the solute concentration *x*, defined in eq 6, at 56.4 MHz. Upper figure (relative to $(CH_3)_4N^+$ internal standard) (a) 9-methylpurine, (b) purine. Lower figure (relative to 9-Me) (c) 9-methylpurine; point above curve c, 0.799 *M* purine plus 0.192 *M* 9-methylpurine.

pH Measurements. pH was measured with a Beckmann research pH meter and a Beckman No. 39030 glass and reference "combination electrode." The meter was calibrated frequently with a standard pH buffer. pH measurements on the reaction mixtures were made directly in the nmr sample tubes, which consisted of a thin-walled 5-mm o.d. glass test tube (to fit into the nmr probe) that was joined at its top onto a 10-ml glass reservoir of adequate size for immersion of pH electrodes. pH measurements for pK_A determinations were made in a hollow-walled beaker through which thermostated water was circulated. In the calculations of pK_{A1} and pK_{A2} from the pH-titration data, exact formulas for a dibasic acid were used. As a check on this method for determining pK_A, *m*-nitrophenol was treated in the same way and appeared to have a pK_A of 8.28 in 0.1 *N* NaCl at 20° (lit.[11] 8.41 at zero ionic strength at 20°; predicted 8.20 at 20° and 0.1 *N* NaCl.)

Nmr Measurements. Rates of proton exchange were derived from nmr measurements of T_2 and T_1 of the "dominant line" in the purine NH-water OH proton system. The nmr spectrometer operated at 56.4 MHz. Details have been described in previous publications from this laboratory.[8,9]

Rates of NH-OH proton exchange were calculated from the exchange broadening, Δ. In the present system, it was necessary to measure (a) $[(1/T_2 - (1/T_1)]$ for purine in 0.1 *N* NaCl at the desired pH, and (b) $[(1/T_2 - (1/T_1)]$ for 0.1 *N* NaCl at the same pH. Δ was taken as the difference, $\Delta = [(1/T_2) - (1/T_1)]_{\text{PuH. NaCl}} - [(1/T_2) - (1/T_1)]_{\text{NaCl}}$, at the given pH.

Measurement b is necessary because Meiboom has shown that near pH 7 there is some exchange broadening from proton exchange between water molecules, even in water containing $H_2{}^{17}O$ at natural abundance.[12] The relative magnitudes of results obtained in a and b and of Δ are shown in Figure 1.

The calculation of the rate of proton exchange *R* from Δ makes use of eq 5, discussed previously,[8b] where $p = [\text{PuH}]/([\text{PuH}] + 2[H_2O])$ $\tau = [\text{PuH}](1 - p)/R$, and δ is the NH-OH proton chemical shift. For physically real values of Δ/*p*, eq 5 has two real roots, one

$$\frac{\Delta}{p} = \frac{\delta^2\tau}{1 + \delta^2\tau^2} \qquad (5)$$

with $\delta\tau > 1$, the other with $\delta\tau < 1$. By raising the temperature at constant composition and finding that Δ always decreases, we conclude that the root $\delta\tau < 1$ is always the appropriate one in the present system. This implies, unfortunately, that proton exchange is always so fast that the NH and OH proton resonances are collapsed into a single line and cannot be observed as separate resonances. Thus δ cannot be measured directly.

Chemical Shifts. To measure δ indirectly, we used a solvent consisting of 0.1 *N* $(CH_3)_4NCl$, rather than 0.1 *N* NaCl, in water. We then measured chemical shifts (relative to $(CH_3)_4N^+$ as internal standard) for (a) the H_2O proton resonance in the presence of 9-methylpurine (m^{9}Pu) at several concentrations, and (b) the collapsed H_2O-NH proton resonance in the presence of purine at various concentrations. Results are plotted in the upper part of Figure 2. The abscissa, *x*, is defined in (6). Note that $x = p$ when

$$x = ([\text{PuH}] + [\text{m}^9\text{Pu}])/(2[H_2O] + [\text{PuH}] + [\text{m}^9\text{Pu}]) \qquad (6)$$

$[\text{m}^9\text{Pu}] = 0$. In both series a and b the relationship of chemical shift to *x* is linear within the experimental error of ±0.15 Hz.

$$\delta_a = \delta_{OH} = \delta_{OH}{}^0 + S_a x \qquad (7a)$$

$$\delta_b = \delta_{\langle OH-NH \rangle} = \delta_{OH}{}^0 + S_b x \qquad (7b)$$

In series a we use 9-methylpurine as a model substance to evaluate the medium effect on δ_{OH} in the absence of NH protons. 9-Methylpurine is closely similar to purine not only in molecular structure but also in its tendency toward self-association in water.[5] If we assume that "medium effects" of purine and 9-methylpurine at equal *x* are equal, we can analyze series b to obtain δ, as follows (eq 8-10).

$$(\delta_b) = (1 - p)\delta_{OH} + p\delta_{NH} = \delta_{OH} + p\delta \qquad (8)$$

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[11] L. P. Fernandez and L. G. Hepler, *J. Am. Chem. Soc.*, **81**, 1783 (1959).

[12] S. Meiboom, *J. Chem. Phys.*, **34**, 375 (1961).

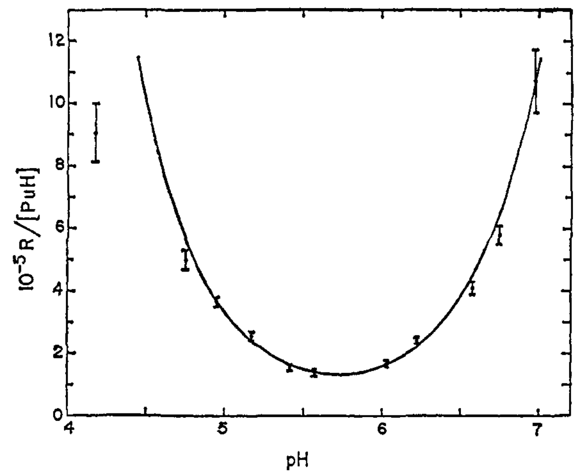

Figure 3. R/[PuH] for 0.85 M purine at 20.4° in 0.1 N aqueous NaCl as a function of pH. Vertical bars represent values calculated from the experimental Δ according to eq 5, as well as their standard deviations. Smooth curve represents eq 3, using appropriate rate constants from Table I.

At equal x, $\delta_{OH} = \delta_a$; therefore

$$(\delta_b)_x - (\delta_a)_x = p\delta \qquad (9)$$

In series b, $p = x$. On utilizing eq 7, we obtain

$$\delta = S_b - S_a \qquad (10)$$

On substituting numerical values for the slopes S_a and S_b, we obtain δ = 680 Hz.

The lower part of Figure 2 shows a similar analysis using the 9-methyl proton resonance as internal standard. The medium effect (curve c) is now quite large and nonlinear, presumably because of self-association.[3] If we assumed again that the medium effect is a colligative function of x, eq 9 suggests the geometrical evaluation of δ that is illustrated in the lower figure. In the experiment represented by the point lying above curve c, x = 0.00887, p = 0.00715, and the vertical displacement (which is equal to $p\delta$) is 5.65 Hz. Thus δ = 780 Hz, not too different from the more reliable result obtained using $(CH_3)_4N^+$ as internal standard. In the calculation of proton exchange rates we have used an average value of 700 Hz (4400 sec^{-1}; 12.4 ppm).

It is instructive to digress for a moment and analyze the lower Figure 2 on a different basis. Let us take δ = 680 Hz from the upper figure and calculate the medium effect on $\delta_{OH}{}^{9-Me}$ at x = 0.00887. When the solute is entirely 9-methylpurine, $(\delta - \delta^0)_{OH}{}^{9-Me}$ = 15.0 Hz, according to curve c. When the solute is largely purine, $(\delta - \delta)_{OH}{}^{9-Me} = 15.0 + 5.56 - 680 \times 0.00715 = 15.7$ Hz. That is, replacement of most of the 9-methylpurine molecules by purine molecules in the molecular stacks has only a minor effect on the 9-methyl proton chemical shift. It follows, with high probability, that the geometry of stacking of the molecules is nearly the same in purine complexes as in 9-methylpurine complexes.

Kinetic Analysis. Figure 3 shows the plot of R/[PuH] *vs.* pH obtained for 0.85 M purine from the data in Figure 1. The calculations are based on eq 5 and δ = 4400 sec^{-1}. The smooth curve represents rate law 3, with rate constants as listed in Table I. In the pH range 4.7–7.0, which includes all but one of the experimental points, the fit is evidently satisfactory. The deviation of the point at pH 4.2 in Figure 3 appears to be significant, but more work is needed to establish its cause. Data obtained at 0.038 M purine are represented by eq 3 comparably well.

Since we were interested in detecting possible effects of molecular complexing on the kinetics at high purine concentrations, we thought, when we started this work, that we should first secure the rate law at low purine concentrations. Our early measurements were of lower precision than those illustrated in the figures, but the pH at which R/[PuH] goes through a minimum could be established to ±0.03 unit. Results, obtained at 30°, are listed in Table II.

Table II. Test of Eq 3 for Proton Exchange of Purine in 0.1 N NaCl in Water at 29.7 ± 0.2°

[PuH], F	$(pH)_{min}$[a]	$(pH)_{min}$ + ½ log [PuH]	$\langle n \rangle$[b]
0.038	6.36	5.65	1.068
0.101	6.15	5.65	1.165
0.185	6.03	5.66	1.276

[a] pH at which R/[PuH] is at a minimum, *i.e.*, Δ is at a maximum.
[b] Number-average degree of association; see eq 2, K = 1.9 at 30°.[2,4]

According to eq 3, as the pH is varied at constant [PuH], R/[PuH] goes through a minimum when $k_{H^+}[H^+] = k_2[Pu^-] = k_2K_{A2}[PuH]/[H^+]$. Hence $(pH)_{min}$ should vary with [PuH] according to

$$(pH)_{min} = {}^1/_2 \log (k_{H^+}/k_2K_{A2}) - {}^1/_2 \log [PuH] \qquad (11)$$

The data in Table II show that $(pH)_{min}$ + ½ log [PuH] is indeed constant, as required.