"Solute-Solvent Interactions", Marcel Dekker, New York, N.Y., 1969; M. H. Abraham, *Prog. Phys. Org. Chem.*, **11**, 1–87 (1974).
(3) J. H. Fendler and E. J. Fendler, "Catalysis in Micellar and Macromolecular Systems", Academic Press, New York, N.Y., 1975.
(4) Y. Pocker and R. F. Buchholz, *J. Am. Chem. Soc.*, **92**, 2075, 4033 (1970); **93**, 2905 (1971).
(5) D. R. Storm and D. E. Koshland, Jr., *Proc. Natl. Acad. Sci. U.S.A.*, **66**, 445 (1970); S. Milstein and L. A. Cohen, *ibid.*, **67**, 1143 (1970).
(6) D. R. Storm and D. E. Koshland, Jr., *J. Am. Chem. Soc.*, **94**, 5805 (1972).
(7) S. Scheiner, W. N. Lipscomb, and D. A. Kleier, *J. Am. Chem. Soc.*, **98**, 4770 (1976).
(8) T. C. Bruice and A. Turner, *J. Am. Chem. Soc.*, **92**, 3422 (1970).
(9) G. Choux and R. L. Benoit, *J. Am. Chem. Soc.*, **91**, 6221 (1969).
(10) C. N. Yiannios and J. V. Karabinos, *J. Org. Chem.*, **28**, 3246 (1963); T. J. Wallace, *J. Am. Chem. Soc.*, **86**, 2018 (1964).
(11) R. N. Haszeldine and J. M. Kidd, *J. Chem. Soc.*, 4228 (1954).
(12) T. Gramstad and R. N. Haszeldine, *J. Chem. Soc.*, 173 (1956).
(13) J. F. Coetzee, J. M. Simon, and R. J. Bertozzi, *Anal. Chem.*, **41**, 766 (1969).
(14) G. L. Ellman, *Arch. Biochem. Biophys.*, **82**, 70 (1959).
(15) S. Hestrin, *J. Biol. Chem.*, **180**, 249 (1949).
(16) E. M. Arnett and C. F. Douty, *J. Am. Chem. Soc.*, **86**, 409 (1964).
(17) K. Yates, J. B. Stevens, and A. R. Katritzky, *Can. J. Chem.*, **42**, 1957 (1964).
(18) D. R. Storm, Ph.D. Thesis, University of California, Berkeley, 1971; P. G. Strange, A. Dafforn, and D. E. Koshland, Jr., unpublished observations.
(19) J. T. Edward and S. L. R. Meacock, *J. Chem. Soc.*, 2000 (1957).
(20) M. Liles, *J. Chem. Soc., Perkin Trans. 2*, 816 (1972).
(21) This observation does not rule out concerted proton transfer in the acid-catalyzed region around 0.1 M acid.
(22) R. H. Boyd, in J. F. Coetzee and C.D. Ritchie, "Solute-Solvent Interactions", Marcel Dekker, New York, N.Y., 1969, p 68.
(23) A. K. Das and K. K. Kundu, *J. Chem. Soc., Faraday Trans. 1*, **69**, 730 (1973).
(24) O. Popovych, *Crit. Rev. Anal. Chem.*, 1, 73 (1970); A. J. Parker, *Chem. Rev.*, **69**, 1 (1969); Von H. Strehlow, W. Knoche, and H. Schneider, *Ber. Bunsenges. Phys. Chem.*, 77, 760 (1973); R. G. Bates in ref 22, pp 46–97.
(25) B. J. Cox and A. J. Parker, *J. Am. Chem. Soc.*, **95**, 402 (1973); R. Alexander, A. J. Parker, J. H. Sharp, and W. E. Waghorne, *ibid.*, **94**, 1148 (1972).
(26) O. Popovych, A. Gibofsky, and D. H. Berne, *Anal. Chem.*, **44**, 811 (1972).
(27) O. Popovych, *Anal. Chem.*, **46**, 2009 (1974).
(28) G. R. Hedwig, D. A. Owensby, and A. J. Parker, *J. Am. Chem. Soc.*, **97**, 3888 (1975).
(29) W. P. Jencks, *Adv. Enzymol.*, **43**, 249–254 (1975).
(30) R. Henderson, *J. Mol. Biol.*, **54**, 341 (1970); J. D. Robertus, J. Kraut, R. A. Alden, and J. J. Birktoft, *Biochemistry*, **11**, 4293 (1972).
(31) J. F. Kirsch and W. P. Jencks, *J. Am. Chem. Soc.*, **86**, 837 (1964).
(32) G. Wallerburg, J. Boger, and P. Haake, *J. Am. Chem. Soc.*, **93**, 4938 (1971).
(33) J. Crosby, R. Stone, and G. E. Lienhard, *J. Am. Chem. Soc.*, **92**, 2891 (1970).
(34) D. S. Kemp and K. G. Paul, *J. Am. Chem. Soc.*, **97**, 7305 (1975); D. S. Kemp, D. D. Cox, and K. G. Paul, *ibid.*, **97**, 7312 (1975).
(35) P. van Eikeren and D. L. Grier, *J. Am. Chem. Soc.*, **98**, 4655 (1976).

# Tautomerism of Neutral and Cationic N-Substituted 4-Aminopyrazolo[3,4-*d*]pyrimidines

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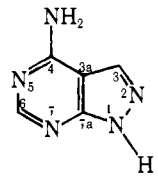
**Abstract:** Neutral 4-aminopyrazolo[3,4-*d*]pyrimidine (4APP) exists in water in two tautomeric forms, 1-H4APP and 2-H-4APP ($K$ = 2-H4APP/1-H4APP = 0.1 at 10 °C, $\Delta H_{\text{tautomerization}}$ = 0.9 kcal mol$^{-1}$). The interconversion of the two forms is catalyzed by $H^+$ and $OH^-$ and proceeds through either an intermediate cation common to both neutral tautomers or through the intermediate anion. $^{13}C$ NMR spectroscopy shows that 1-*i*-Pr4APP (the nontautomerizable model compound for the 1-H4APP tautomer) protonates mainly at N(5). 2-*i*-Pr4APP (model for 2-H4APP) protonates to similar extents at N(5) and N(7). Temperature-jump relaxation confirms this scheme; cation exchanges are catalyzed by $H^+$, $H_2O$ acting as a base, $OH^-$, and the corresponding neutral N-substituted 4APP. It is inferred from the corresponding methylated derivatives that together with the abundant species, 1-H and 2-H4APP, there are small proportions of 7-H4APP ($\simeq 10^{-3}$) and 5-H4APP ($2 \times 10^{-4}$). 7-H4APP exists only as an amino tautomer, whereas 5-H4APP in water has a partial imino structure ([amine]/[imine] = 10); the interconversion of the tautomeric 5-H4APP is catalyzed by $OH^-$, cationic 5-H4APP, and $H_2O$ as proved by the kinetic study of the model compound, 5-Me4APP. Biochemical implications of the location of the basic sites and of the presence of an imino tautomer are tentatively discussed.

The adenine analogue, 4-aminopyrazolo[3,4-*d*]pyrimidine (4APP), has proved to affect nucleotide synthesis in two different ways. It can either bind to adenine phosphoribosyltransferase, an enzyme responsible for the transfer of the phosphoribosyl group from PP-ribose-P to exogenous purine,[1,2]



or it can inhibit purine synthesis *de novo* (from nonpurine components).[3] In the latter case, the mechanism of the inhibition is not clearly understood, but it is suggested[3] that it may arise from the interaction of 4APP with some enzyme involved in the early steps of the reaction. Inhibition of purine synthesis de novo has been considered as the cause of the cytotoxic activity of 4APP. The fixation of adenine phosphoribosyltransferase would involve the electron donor property (the basicity) of the nitrogen atoms at positions 3 and 7 of adenine.[1] It can be thought that the reaction of adenine with the protein which catalyzes synthesis *de novo* will also be dependent on the basicity of the various nitrogen sites. This conclusion can be reconciled with the early empirical rule stating that the effectiveness of purine analogues as cytotoxic drugs is related to their basicities as measured by their p$K_a$ (proton gained), i.e., the closer their values to those of natural purines, the higher the efficiency.[4]

Understanding 4APP activity at the molecular level necessitates, as a starting point, a reexamination of the basicity criterium, not in terms of the *overall basicity*, as evaluated from the p$K_a$, but in terms of the *partial basicity*[5] of the various atoms as determined by the protonation site(s) of the molecule.

Moreover, theoretical considerations suggest that the cytotoxic activity of neighbor compounds aminopyrazolo[4,3-*d*]pyrimidines might arise alternatively from the existence of rare imino tautomers capable of mispairing with cytosine when

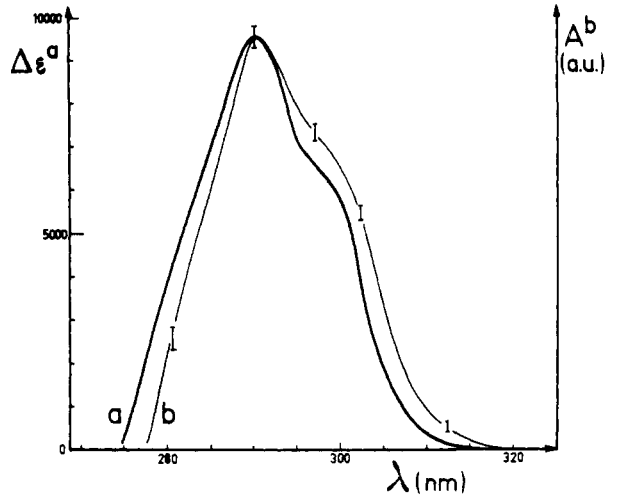



**Figure 1.** (a) Differential spectrum: variation of the quantity $([\epsilon_{4APP} - \epsilon_{2\text{-Me4APP}}])_\lambda$ with the wavelength λ. (This parameter was preferred over $(\epsilon_{1\text{-Me4APP}} - \epsilon_{2\text{-Me4APP}})$ owing to the important shift (6 nm) of 1-substituted 4APP upon methylation.) (b) Ratio of the relaxation amplitude to the 4APP concentration vs. the wavelength: $t_i$ = 1 °C, $t_f$ = 10 °C; uncertainties are the standard deviations.

incorporated in the DNA chain.[6] So far this hypothesis has not been verified experimentally. This hypothesis deserves closer consideration requiring the utmost care (at least for pyrazolo[3,4-*d*]pyrimidine derivatives) since the incorporation (if there is any) of 4APP has not proved to be *causally linked* to its cytotoxic property.[7]

The work we undertook on 4APP was consequently designed to (a) determine the basic site(s) where 4APP protonates and the comparison with the basic sites of adenine, (b) characterize the hypothetical rare tautomer forms, together with the estimation of their *thermodynamic and kinetic* stabilities.

$^{13}C$ NMR spectroscopy is the obvious technique to answer the question of cation structure, since protonation of a nitrogen atom in heterocycles markedly affects the chemical shifts of the neighboring carbons.[8,9] However, the assignment of the resonance lines is likely to be difficult because of the expected existence of several tautomers for both neutral and cationic 4APP, N(1)-H and N(2)-H tautomers supposedly being the main forms of neutral 4APP (as can be inferred from the observation of N(1)-H ⇌ N(2)-H tautomeric equilibrium in pyrazolo[4,3-*d*]pyrimidines).[10,11] Consequently, prior to the NMR study, the occurrence of various tautomers in aqueous solutions of 4APP must be established and an estimate of the relative proportions of these forms must be given. The temperature-jump relaxation technique has proved particularly well fitted in solving this type of problem.[12-14]

## Experimental Section

**Materials.** 4-Aminopyrazolo[3,4-*d*]pyrimidine (Aldrich) was recrystallized from water.

1-Methyl-4-aminopyrazolo[3,4-*d*]pyrimidine[15] and 1,5-dimethyl-4-aminopyrazolo[3,4-*d*]pyrimidine[16] were prepared according to the literature.

1-Isopropyl-, 2-isopropyl-, and 2-methyl-4-aminopyrazolo[3,4-*d*]pyrimidine were a gift from Ciba-Geigy; 2-methyl-4-aminopyrazolo[3,4-*d*]pyrimidine was also prepared according to a published procedure.[17]

5-Methyl-4-aminopyrazolo[3,4-*d*]pyrimidine was prepared by a procedure derived from that quoted in ref 16 where ammonia saturated alcoholic solution was used instead of alcoholic methylamine solution ($T_f$ = 240 °C; mol wt 149 as measured by mass spectroscopy).

7-Methyl-4-aminopyrazolo[3,4-*d*]pyrimidine was prepared as follows. 4APP was stirred in dimethylacetamide with methyl iodide. The course of the reaction was followed by TLC (silica gel). Amounts of $CH_3I$ were added until the reaction was completed. Two products were obtained with only slightly different $R_f$. The reaction mixture was then made basic by addition of aqueous sodium hydroxide and heated. TLC showed, upon heating, the progressive transformation of one of the two initial compounds to a new product with a larger $R_f$. When the reaction was completed the two products were extracted by methanol from the silica plate. The UV spectrum of the product with the large $R_f$ is identical with that of 4-methylaminopyrazolo[3,4-*d*]pyrimidine as prepared by Dimroth transposition of authentic 5-methyl-4-aminopyrazolo[3,4-*d*]pyrimidine. The other compound is 7-methyl-4-aminopyrazolo[3,4-*d*]pyrimidine ($T_f$ = 252 °C; mol wt 149).

For 6,7-dihydro-8-methylimidazo[2,1-*f*]pyrazolo[3,4-*d*]pyrimidine, the procedure used was similar to that described for the preparation of the corresponding adenine[18] ($T_f$ = 265 °C; mol wt 175).

**Kinetic Apparatus and Data Processing.** Temperature-jump experiments were performed as described elsewhere.[12-14] The standard temperature conditions were $t_{initial}$ = 1 °C and $t_{final}$ = 10 °C for kinetic runs in water, and $t_{initial}$ = 4 °C, $t_{final}$ = 10 °C in deuterium oxide.

Actual concentrations of the various species present in solution at $t_{final}$[12] were computed from the pH values at $t_{initial}$ and from the p$K$ values at 20 °C; when not available, the ionization enthalpies were roughly estimated as reported by Katritzky at al.[19]

**pH and p$K$ Measurements.** pH was monitored by a Radiometer PHM64 pH meter equipped with a Radiometer G202C glass electrode. pDs were calculated from pHs using the relationship pD = pH + 0.4, the pH meter being standardized in water.[20]

p$K$ values of new compounds were measured as previously described.[12] p$K_a$'s (proton gained) in $D_2O$ were estimated by the relationship $pK_a(D_2O) = pK_a(H_2O) + 0.5$.

**UV Spectra.** The UV spectra were recorded on a Cary 118 UV spectrophotometer fitted with thermostated cells. Spectra were run in buffer solutions to allow a single species to be present.

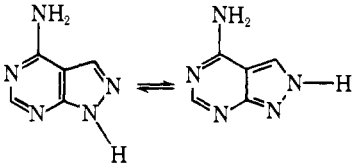
**IR Spectra.** IR spectra of 7-methyl-4-aminopyrazolo[3,4-*d*]pyrimidine and 5-methyl-4-aminopyrimidine were recorded with a Perkin-Elmer 225 IR spectrometer, using Infrasil cells (optical length 2 cm) containing saturated solutions in $CHCl_3$ (Baker GC grade or Fluka).

**$^{13}C$ NMR Spectra.** $^{13}C$ NMR spectra of 1 N aqueous solutions of 1-isopropyl-4-aminopyrazolo[3,4-*d*]pyrimidine and 2-isopropyl-4-aminopyrazolo[3,4-*d*]pyrimidine and their corresponding monocations were recorded with a JEOL PS100/PFT 100 at 25 MHz using 8-mm tubes. $Me_2SO$-$d_6$ (70%) and $Me_2SO$ (30%) contained in an inner tube (1.5 mm) were used as lock and external reference, respectively.

## Results and Discussion

**N(1)H-N(2)H Tautomeric Equilibrium.** A solution of 4APP submitted to temperature jump shows a relaxation phenomenon. The relaxation amplitude is proportional to the initial 4APP concentration, depends on the wavelength of the spectrophotometric detection, and is independent of the pH until it approaches the p$K$ values; this means that the relaxation is related to an equilibrium involving *neutral forms* of 4APP.

Attribution of the relaxation to the equilibrium I is easily



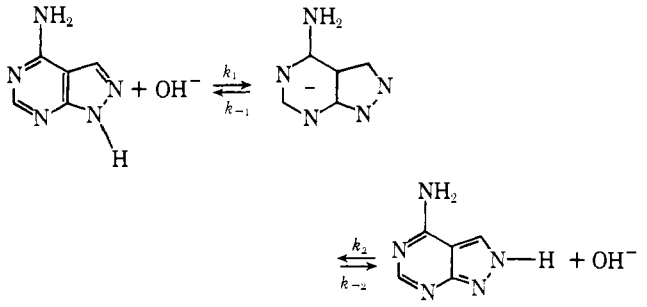

(I)

done by comparing the wavelength dependence of the relaxation amplitude, $A_\lambda$, to the differential spectrum of 1-methyl-4-aminopyrazolo[3,4-*d*]pyrimidine (1-Me4APP) and 2-methyl-4-aminopyrazolo[3,4-*d*]pyrimidine (2-Me4APP), which are the *nontautomerizable model compounds* for 1-H4APP and 2-H4APP (Figure 1).[12,13]

The enthalpy of the equilibrium is readily estimated from the variation of the quantity $A_\lambda T^2$ with temperature to be 0.9 ± 0.1 kcal mol$^{-1}$.[13,21] Replacing $\Delta H$ in the expression of $A_\lambda$ quoted in ref 12 and 13 leads to [2-H4APP]/[1-H4APP] =

0.1. The observed relaxation time is strongly pH dependent but is not significantly affected by the concentration of 4APP between $10^{-4}$ and $2 \times 10^{-3}$ mol $L^{-1}$ (Figure 2).

This means that the tautomeric interconversion proceeds via an intermediate *ionic form* of 4APP (cation or anion) and the concentration of this ionic intermediate is so low that no autocatalytic process occurs, at least in our concentration range. The anion concentration is always very low since our highest pH value (around 9) lies far below the $pK_b$ (proton lost) of 4APP (11.03 at 20 °C). However, the cation concentration in the acidic pH range (the lowest pH value is 5.3), given $pK_a$ = 4.67 at 10 °C, is not negligible (more than 10% of the total 4APP concentration). The fact that no autocatalytic effect is observed, despite this important cation concentration, implies that the intermediate cation in the tautomerization process *is not* by far the major positively charged species of protonated 4APP. The kinetics of tautomerization in the basic pH range (pH >8) is readily understood in terms of the following base-catalyzed mechanism.



Applying the steady state approximation to the intermediate anion yields the following expression for the relaxation time:

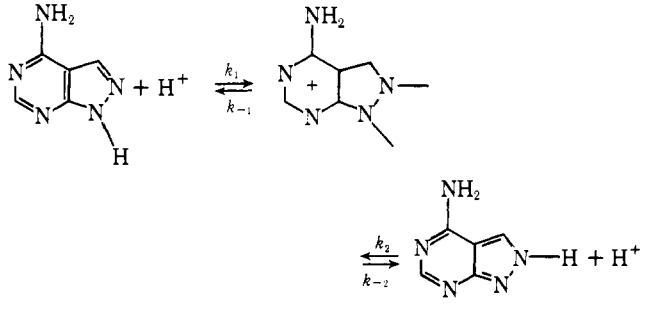
$$(\tau_b)^{-1} = \frac{k_1 k_{-2} + k_{-1} k_2}{k_{-1} + k_{-2}} [OH^-] \qquad (1)$$

which may be rewritten[22] as

$$(\tau_b)^{-1} = k_1 [OH^-] \qquad (1')$$

Log $(\tau_b)^{-1}$ should be a linear function of the pH with a slope equal to unity. This agrees excellently with the experimental observations (Figure 2).

Fitting the experimental data in the basic pH range with eq 1′ gives $k_1 = (1.08 \pm 0.3) \times 10^{10}$ $M^{-1}$ $s^{-1}$ (Table I). The absence of any significant pH-independent term in the linear fitting establishes the direct transfer of the proton between the N(1) and N(2) nitrogen atoms does not occur despite their proximity. This is consistent with our previous kinetic study of 3-phenyl-5-methylpyrazole tautomerism. At acidic pHs (pH <7), the mechanism of tautomerization is likely to be



The relaxation time related to this pathway is then[22]

$$(\tau_a)^{-1} = k_1 [H^+] \qquad (2)$$

This equation does not account well for the experimental observations, since plotting the experimental values of log $(\tau_a)^{-1}$ vs. pH leads to a straight line whose slope *differs* slightly

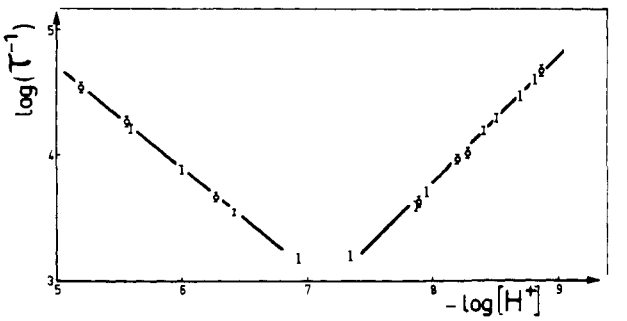



Figure 2. Neutral 4APP: log $\tau^{-1}$ ($s^{-1}$) as a function of $-\log [H^+]$; concentrations $6.3 \times 10^{-4}$ (I) and $1.1 \times 10^{-3}$ mol $L^{-1}$ (Q).

but significantly from unity (Figure 2). The experimental data are conversely well fitted by the linear relationship

$$(\tau_a)^{-1}_{4APP} = k_{H+}[H^+] + k_0 \qquad (2')$$

where $k_{H+} = (6.0 \pm 0.2) \times 10^9$ $M^{-1}$ $s^{-1}$ and $k_0 = (1300 \pm 200)$ $s^{-1}$.

This slight discrepancy is understood in terms of the presence at acidic pHs of appreciable amounts of cationic 2-H4APP, whose $pK_a$, as inferred from that of 2-Me4APP, should be about 5.1 at 20 °C.

The low experimental value of the rate of proton fixation, $k_{H+}$, is consistent with a very low concentration of the common cation N(1)H-N(2)H as resulting from an expected low value of its $pK_a$ of formation;[23] thus, the formation of this particular cation would be only slightly exothermic and the fixation of a proton would no longer be diffusion limited.[14] We shall admit, however, that the protonation occurs at the *same rate* at N(1) and N(2) atomic sites as per the previous hypothesis.[22]

**$^{13}$C NMR Study.** The nontautomerizable 1-isopropyl-4-aminopyrazolo[3,4-*d*]pyrimidine (1-*i*-Pr4APP) and 2-isopropyl-4-aminopyrazolo[3,4-*d*]pyrimidine (2-*i*-Pr4APP), which are the model compounds for 1-H4APP and 2-H4APP tautomers, were preferred for the NMR study over the corresponding methylated derivatives owing to the high solubility in water of their neutral forms (1 N concentrations are easily obtained). The $^{13}$C NMR spectra were recorded at room temperature. The attribution of the resonance lines was achieved by off-resonance decoupling or undecoupling (Table II), and for neutral 1-*i*-Pr4APP and 2-*i*-Pr4APP, is consistent with previously reported data.[24]

**Protonation of 1-*i*-Pr4APP.** Upon protonation, C(4) and C(6) carbon atoms of 1-*i*-Pr4APP undergo a strong upfield shift of 8.38 and 8.46 ppm, respectively, while C(3) and C(7a) shift downfield by 3.4 and 1.9 ppm (Table II). As definitely established on numerous examples by Grant et al.,[8,9] the protonation of a ring nitrogen atom induces an upfield shift on the adjacent carbon atoms ($\alpha$) by 6-11 ppm (at least when no tautomeric equilibrium is involved). Hence, 1-*i*-Pr4APP is protonated mainly at the N(5) site. However, one can postulate that protonation in water may occur to a low extent on the N(7) nitrogen atom, as is suggested by a rather weak downfield shift of C(7a) as compared to that of C(3), both of which might result from averaging of signals from the N(5) and N(7) protonated species.[25] Though somewhat farfetched, this hypothesis is partially substantiated by protonation experiments on 1-*i*-Pr4APP in $Me_2SO$ (Table II). The positive (upfield) protonation shift at C(4) is now significantly lower than at C(6) and the shift at C(7a) now becomes slightly *positive* (whereas negative in water), as expected from the occurrence of a downfield effect at C(4) and an upfield effect at C(7a) due to protonation at N(7). This suggests that protonation at N(7) occurs in $Me_2SO$ to a larger extent than in water. New evi-

**Table I.** Kinetic Rate Constants for Tautomeric Interconversion of Neutral and Cationic N-Substituted 4APP

| Compd | $k_{H^+}$, $M^{-1}s^{-1}$ | $k_{OH^-}$, $M^{-1}s^{-1}$ | $k_A$ (autocatalysis), $M^{-1}s^{-1}$ | $k_0$, $s^{-1}$ |
|---|---|---|---|---|
| 4APP | $5.8 \times 10^9$ (0.2)[b] | $1.1 \times 10^{10}$ (0.3) | | |
| 2-*i*-Pr4APP, cationic[a] | $5.5 \times 10^8$ (0.13) | | $1.0 \times 10^8$ (0.04) | $2.4 \times 10^4$ (0.6) |
| 7-Me4APP, cationic | $1.2 \times 10^8$ (0.13) | $2. \times 10^{10}$ (0.8) | $1.43 \times 10^8$ (0.35) | $1.92 \times 10^4$ (0.12) |
| 5-Me4APP, cationic | $3.0 \times 10^8$ (0.5) | $1.5 \times 10^{10}$ (0.6) | $1.6 \times 10^8$ (0.3) | $1.03 \times 10^3$ |
| 5-Me4APP[a] | | $9.8 \times 10^9$ (0.7) | $1.82 \times 10^8$ (0.2) | $1.12 \times 10^4$ (0.37) |

[a] In $D_2O$. [b] Standard deviation.

**Table II.** $^{13}C$ NMR Values of Chemical Shifts of Neutral and Cationic 1-*i*-Pr4APP and 2-*i*-Pr4APP

A. 1-Isopropyl-4APP

| | δ ppm (neutral)[a] | | δ ppm (cation) | | Δδ, ppm[b] | |
|---|---|---|---|---|---|---|
| Position | $H_2O$ | $Me_2SO$ | $H_2O$ | $Me_2SO$[c] | $H_2O$ | $Me_2SO$ |
| 3 | −90.6 | −91.01 | −94 | −94.82 | −3.4 | −3.81 |
| 3a | −58.7 | −59.8 | −58.56 | −58.94 | +0.14 | +0.86 |
| 4 | −116.53 | −117.62 | −108.15 | −108.88 | +8.38 | +8.74 |
| 6 | −113.85 | −115.05 | −105.39 | −105.93 | +8.46 | +9.12 |
| 7a | −109.08 | −111.64 | −110.98 | −111.21 | −1.9 | +0.43 |
| $CH_{i\text{-Pr}}$ | −8.12 | −7.61 | −9.54 | −9.13 | −1.47 | −1.52 |
| $CH_3$ | 19.73 | 18.52 | 19.57 | 18.48 | +0.16 | ~0 |

B. 2-Isopropyl-4APP

| Position | δ (neutral)[a] | δ (cationic) | Δδ[b] |
|---|---|---|---|
| 3 | −82.24 | −87.37 | −5.13 |
| 3a | −59.06 | −58.24 | +0.82 |
| 4 | −116.69 | −113.0 | +3.69 |
| 6 | −114.63 | −106.08 | +8.55 |
| 7a | −117.89 | −114.24 | +3.65 |
| $CH_{i\text{-Pr}}$ | −14.95 | −16.32 | −1.37 |
| $CH_3$ | 18.91 | 18.83 | ~0 |

[a] Reference $Me_2SO$. [b] Δδ = (cation) − δ (neutral). [c] 1-*i*-Pr4APP HCl is dissolved in $Me_2SO$. It is admitted that no deprotonation occurs.

dence for N(7) protonation of 1-*i*-Pr4APP based on the $pK_a$ value of N-substituted 4APP and on the kinetic data of prototropic transfer occurring in their cations is presented further on.

**Protonation of 2-*i*-Pr4APP.** Protonation of 2-*i*-Pr4APP in water leads to a positive shift at C(6) of 8.5 ppm, while C(4) and C(7a) are *both positively shifted* by 3.7 ppm (Table II). This strongly suggests that 2-*i*-Pr4APP protonates to similar extents at N(5) and N(7) and, consequently, that in acidic aqueous solutions it exists as a mixture of roughly identical proportions of 2-*i*-Pr-5-H-APPium-2-*i*-Pr-7-H-APPium.

We now present a tentative temperature-jump confirmation of the hypothesis relative to the various tautomeric cations of 1- and 2-*i*-Pr4APP. For the sake of clarity, we start by investigating the equilibrium between the cations of 2-*i*-Pr4APP, the standing model compound for 2-H4APP—itself the minor tautomer of 4APP.

**Kinetics of 2-*i*-Pr4APP Cation Exchange.** An aqueous solution of cationic 2-*i*-Pr4APP (cf. $pK_a$ values in Table III) exhibits a relaxation spectrum after undergoing a fast temperature jump. The relaxation time is pH dependent and, when the pH value is above 4.7, it depends on the initial 2-*i*-Pr4APP concentration. The invariance of the relaxation is associated with an equilibrium involving two monocationic species which, as seen above, are likely to be 2-*i*-Pr-5-H-4APPium ($C_{2,5}$) and 2-*i*-Pr-7-H-4APPium ($C_{2,7}$).[26] The relaxation time is very short and never exceeds 10 μs. Such a time scale lies in the limit of accurate functioning of our experimental temperature-jump assembly; thus, the inaccuracy of relaxation time values is large. This difficulty is easily overcome by performing the temperature-jump experiment in $D_2O$ in order to decrease the reaction rates. The same kinetic pattern as in $H_2O$ is obtained (Figure 3). Since the relaxation time depends on pD and neutral 2-*i*-Pr4APP concentration, it is accounted for by the relationship

$$(\tau^{-1})_{D_2O} = k_{D^+}[D^+] + k_A[2\text{-}i\text{-Pr4APP}] + k^0{}_{D_2O} \quad (3)$$

where [2-*i*-Pr4APP] is the actual concentration of neutral 2-*i*-Pr4APP at the considered pD. The contribution of a catalysis by $OD^-$ is not significant in the pD range of the study. A multilinear fitting leads to the values quoted in Table I. One should note the large value of the $k^0{}_{D_2O}$ term which arises from a relaxation route independent of pD and accounts for the less than unity slope when plotting log $(\tau^{-1})_{D_2O}$ vs. pD (Figure 3).

The following simple mechanism accounts well for the observed results.

**(a) Acid Catalysis (pD <4.5).** This relaxation pathway implies the presence of an intermediate dication:

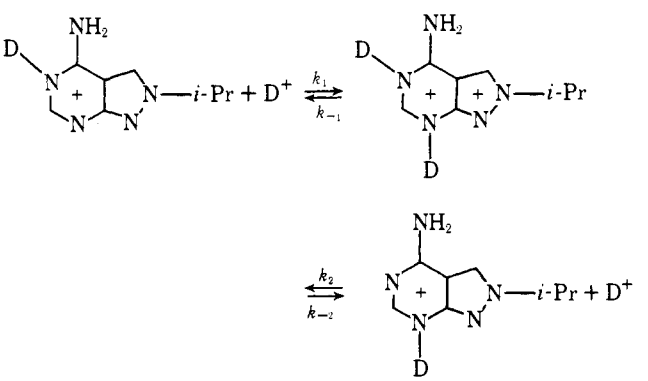


**Table III.** p$K$ Values of Substituted 4APP

| | 4APP | I-Me4APP | 2-Me4APP | 2-*i*-Pr4APP | 5-Me4APP | 1,5diMe4IPP | 7-Me4APP | 6,7-diH-8MePP |
|---|---|---|---|---|---|---|---|---|
| p$K_a$[a] | 4.61 (0.03) | 4.30 (0.03) | 5.11 (0.02) | 5.35 (0.03) | 8.08 (0.02) | 8.57 (0.04) | 7.13 (0.03) | 8.05 (0.03) |
| p$K_b$ (proton lost) | 11.03 (0.03) | | | | 11.38 (0.02) | | | |

[a] Values measured in water solution of ionic strength equal to 0.1, at 20 °C.

An expression of the relaxation time at acidic pH values is readily obtained:[22]

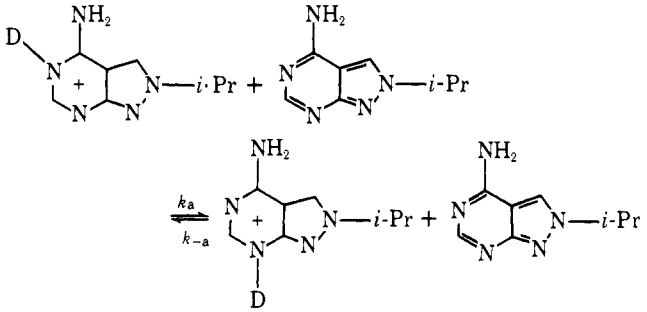
$$(\tau)_a^{-1} = k_1[D^+] \quad (4)$$

where $k_1$ is the second-order rate constant for $D^+$ fixation on the monocations.

The existence of a second acidic p$K$ of 2-*i*-Pr4APP is confirmed by the spectral modifications observed when the pH of aqueous solutions of 2-*i*-Pr4APP is varied from 1 to 0 (p$K \simeq$ 0.5 at 20 °C); though not observed in 4APP itself (presumably because of close spectral similitudes of mono- and dications), dication formation at low pH may, however, be reasonably assumed. The value of $k_{D^+}$ from eq 3 (Table I) appears to be somewhat lower than the classical proton fixation rate constant. This is understood from the facts that (a) the proton fixes on a *positively charged* species, (b) the p$K$ of dication formation being near zero, the protonation is only slightly thermodynamically favorable and is no longer diffusion controlled.

**(b) Base Catalysis (pD >5).** In the experimental pD range, the only significant basic catalysts are likely to be neutral 2-*i*-Pr4APP and $D_2O$.

**(1) Catalysis by Neutral 2-*i*-Pr4APP (Autocatalysis).** The reaction scheme is



Assuming that the concentration of neutral 2-*i*-Pr4APP is small compared to that of the cations and that it is constant, the relaxation time for this route is

$$(\tau)^{-1}_{auto} = (k_a + k_{-a})\frac{K_a C_0}{K_a + [D^+]} \simeq (k_a + k_{-a})\frac{K_a C_0}{[D^+]} \quad (5)$$

where $K_a$ is the equilibrium constant of monocation formation from neutral 2-*i*-Pr4APP (5.85 at 20 °C in $D_2O$) (Table III) and $C_0$ the analytical concentration of 2-*i*-Pr4APP.

The value of the autocatalytic rate constant, $k_A$, from eq 3 (Table I) compares well with autocatalytic rate constants observed in tautomerizable purines and pyrimidines in water ($k_{adenine/adeninate} = 3.5 \times 10^8$ $M^{-1}$ $s^{-1}$, $k_{cytosine/cytosinium} = 4.4 \times 10^8$ $M^{-1}$ $s^{-1}$) and in $D_2O$ ($k_{3\text{-Mecytosine}/3\text{-Mecytosinium}} = 2.2 \times 10^8$ $M^{-1}$ $s^{-1}$).[12,13]

**(2) Catalysis by $D_2O$.** The pH-independent catalytic pathway for cation interconversion (whose contribution, in the kinetic law (3), is represented by the term $k^0_{D_2O}$ (Table I)) deserves special attention. It may arise either from a direct *nondissociative* proton transfer from the N(5) nitrogen atom to the N(7) atom, or from catalysis by $D_2O$ acting as a base, thereby implying a dissociative step. The reaction according to the latter mechanism will proceed as follows:

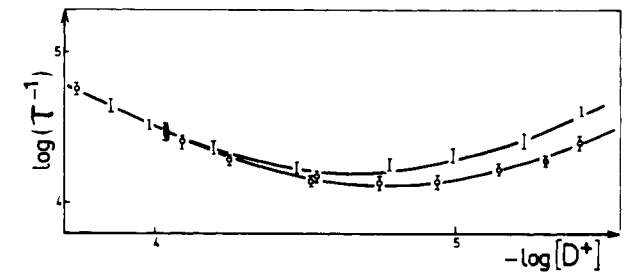
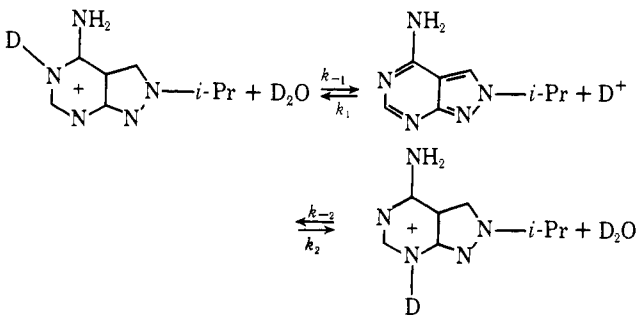



Figure 3. Cationic 2-*i*-Pr4APP: log $\tau^{-1}$ ($s^{-1}$) as a function of $-\log[D^+]$; concentrations $4.53 \times 10^{-4}$ (Q) and $7.2 \times 10^{-4}$ mol $L^{-1}$ (I).



The conventional treatment leads to[12,14,22]

$$(\tau)^{-1}_{D_2O} = \frac{k_{-1} + k_{-2}}{2} \quad (6)$$

$k_{-1}$ and $k_{-2}$ can be expressed in terms of the equilibrium constant of cation formation, $K_a$:

$$K_a = \frac{[2\text{-}i\text{-Pr4APP}][H^+]}{\Sigma \text{ cations}}$$

and

$$\frac{1}{K_a} = \frac{1}{K_{C_{2,5}}} + \frac{1}{K_{C_{2,7}}} \quad (7)$$

where $K_{C_{2,5}}$ and $K_{C_{2,7}}$ are the formation constants of cations $C_{2,5}$ and $C_{2,7}$, which may be expressed as a ratio of rate constants: $K_{C_{2,5}} = k_{-1}/k_1$ and $K_{C_{2,7}} = k_{-2}/k_2$; the diffusion rate constants $k_1$ and $k_2$ being equal, $(\tau)^{-1}_{D_2O}$ is rewritten as

$$(\tau)^{-1}_{D_2O} = \frac{k_1}{2}[K_{C_{2,5}} + K_{C_{2,7}}] \quad (6')$$

Now, if we express the equilibrium constant between the tautomeric cations, $K_4 = [C_{2,5}]/C_{2,7} = K_{C_{2,7}}/K_{C_{2,5}}$, we see that as $K_4$ decreases, $K_{C_{2,5}}$ becomes larger. This means that the relaxation time $(\tau)^{-1}_{D_2O}$ tends to be controlled by the acidity constant of the *less abundant* tautomer. This conclusion has been checked many times against experimental data and has proved to be quite valid.[13,14]

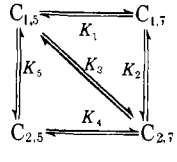
The experimental value of $k^0_{D_2O}$ from eq 3, together with the hypothesis of similar proportions of $C_{2,5}$ and $C_{2,7}$ as inferred from $^{13}C$ NMR, leads to a value of the formation constant of one individual cation p$K_{C_{2,5}}$ = p$K_{C_{2,7}}$ = 5.6 at 10 °C in $D_2O$ (with $k_1$ taken equal to $10^{10}$ $M^{-1}$ $s^{-1}$). In water, p$K_{C_{2,5}}$

or $pK_{C_{2,7}}$ will be 5.1 at 10 °C, a value which compares very satisfactorily with that obtained from the $pK_a$ of 2-*i*-Pr4APP (5.35 at 20 °C); this means, with equal cation proportions, that $pK_{C_{2,5}} = pK_{C_{2,7}} = 5.05$ at 20 °C (Table III). The fact that the exchange of $C_{2,5}$ and $C_{2,7}$ is well accounted for solely by the water catalysis mechanism allows ruling out the occurrence of a significant direct proton transfer. Similar conclusions already appear in this paper for the tautomerism of neutral 4APP as well as for neighboring systems (such as tautomeric cytosine, isocytosine, and pyridinols) where no nondissociative proton exchange is observed.[13,14]

**Kinetics of 1-*i*-Pr4AAP Cation Exchange.** Simple considerations show that no kinetic information about this tautomeric system (if it exists) can be obtained with our temperature-jump spectrometer. The $pK_a$ of 1-*i*-Pr4APP is expected to be around 4.8 at 20 °C (as inferred from the p*K* of 1-Me4APP). Moreover, if the $^{13}C$ NMR conclusions are valid, 1-*i*-Pr-7-H-4APPium ($C_{1,7}$) is present in a low percentage proportion and, hence, has a $pK_a$ value around 4. The relaxation time will be expressed by a relationship, similar to eq 3, in which the $k^0$ term would be around $10^6$ $s^{-1}$. This corresponds to a relaxation time for the cation interconversion of less than 1 μs, which is too short a time to be studied with our apparatus. The relaxation signal of cationic 1-*i*-Pr4APP we observed indeed has a time constant which cannot be discriminated from that of the heating (3 μs). However, the study of the 1-*i*-Pr4APP cation exchange can be indirectly achieved. The 7-methyl-4-aminopyrazolo[3,4-*d*]pyrimidine (7-Me4APP) has a $pK_a$ of 7.2 at 20 °C (Table III) and is likely to protonate mainly at the N(2) site because protonation at that site leads to the *same stable cationic* structure as that from protonation of 2-*i*-Pr4APP at N(7).

In generalizing this concept we shall formulate the hypothesis that cations substituted at N(2) and N(5) ($C_{2,5}$) have a stability comparable to that of cations substituted at N(2) and N(7) ($C_{2,7}$) (cf. above) *whatever the substituent* (H, Me, *i*-Pr). Thus, the relative stability of a cation with the structure $C_{1,7}$ (such as the hypothetical *rare tautomeric* cation of 1-*i*-Pr4APP) toward a cation with the structure $C_{1,5}$ is easily related to its relative stability toward a cation with the $C_{2,7}$ structure. Thus, if the main cation of 1-*i*-Pr4APP coexists with small proportions of $C_{1,7}$ cationic tautomer, the main cation of 7-Me4APP, which has the structure $C_{2,7}$, must be present together with predictable amounts of $C_{1,7}$ cation. This means that observing and studying the tautomeric equilibrium between the cations of 7-Me4APP will lead to conclusions applying to the equilibrium of the tautomeric cations of 1-*i*-Pr4APP. The advantages of doing so are obvious: the $pK_a$ of 7-Me4APP (Table III) exceeds the $pK_a$ of 1-*i*-Pr4APP and 1-Me4APP by almost 2 units and even if the rare tautomeric cation is present in a low percentage proportion, its $pK_a$ will be high enough so that the $k^0$ term in the kinetic law will be sufficiently weak to permit the measurement of the relaxation time with our apparatus.

This procedure is summarized in the following scheme:




Equilibrium constant $K_1$ is easily deduced from $K_2$ (which will now be estimated from the study of cationic 7-Me4APP) and from $K_3$ obtained from the $pK'_a$s of 2-Me4APP and 1-Me4APP with the equilibrium constant [2-H4APP]/[1-H4APP] = 0.1. It is noteworthy that since we have shown that $C_{2,7}$ is present with similar proportions of the $C_{2,5}$ species, an equilibrium between $C_{1,5}$ and $C_{2,5}$ ($K_5$) *must obviously* take place. This hypothesis will be checked by the study of the exchange of 5-Me4APP cations (which have the structures $C_{5,2}$ and $C_{5,1}$ whatever the actual tautomeric form of neutral 5-Me5APP). This study appears herein after our examination of 7-Me4APP cation tautomerism.

**7-Me4APP Cation Exchange.** The variation of the observed relaxation time with pH is well fitted by a law similar to eq 4 (Table I):

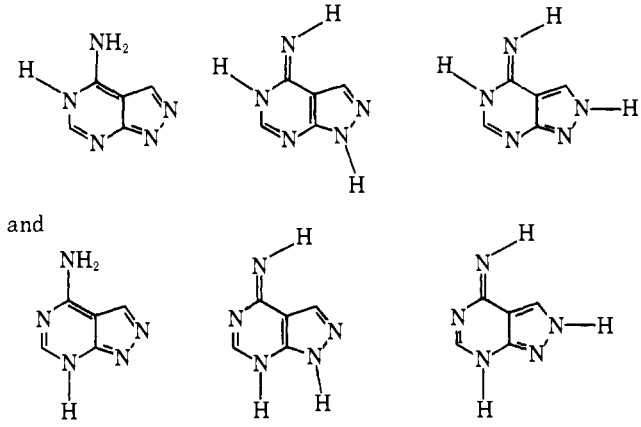
$$(\tau^{-1}) = k_{H^+}[H^+] + k_{OH^-}[OH^-] + k_A[\text{7-Me4APP}] + k^0_{H_2O} \quad (8)$$

This is quite consistent with a catalytic mechanism similar to that of the interconversion of cationic 2-*i*-Pr4APP species, except that, here, the contribution of catalysis by $OH^-$ must be taken into account. The value of $k^0_{H_2O}$ allows the determination of the $pK_a$ of the rare tautomer (when assuming the absence of direct proton transfer) estimated to be 5.4 at 10 °C. The proportion of the rare $C_{1,7}$ cation is hence readily estimated to be a few percent of the total cationic species. We can reasonably estimate the equilibrium constants: $K_1 = C_{1,7}/C_{1,5} = 2 \times 10^{-2}$; $K_2 = C_{1,7}/C_{2,7} = 5 \times 10^{-2}$; $K_3 = C_{2,7}/C_{1,5} = 0.3$; $K_4 = C_{2,5}/C_{2,7} = 1$; $K_5 = C_{2,5}/C_{1,5} = 0.3$.

It should be stressed that these values cannot claim to by anything other than rather rough estimates of the real ones. The fact that $K_1$ has the same order of magnitude as the value quoted in ref 25 must be considered as satisfactory enough.

**5-Me4APP Cation Exchange.** The variation of the relaxation time with pH follows the expected law which contains both acidic catalysis by $H^+$ involving an intermediate dication and basic catalysis by $OH^-$ and neutral 5-Me4APP (Table I). Owing to the high value of the $pK_a$ (Table III), catalysis by water is expected to be insignificant and, in fact, log $(\tau^{-1})$ vs. pH is a straight line whose slope is unity.

**Rare Tautomers of 4APP.** From the $pK_a$ values of the various N-methylated derivatives of 4APP, together with the knowledge of their protonation sites and the proportions of the tautomeric cations, one readily sees that, besides the abundant tautomers 1- and 2-H4APP,[27] there are small proportions of 5- ($2 \times 10^{-4}$) and 7-H4APP ($\simeq 10^{-3}$) which obviously are not directly observable. This raises the important question of the *actual structure* of these rare forms in terms of amine-imine tautomerism:




Neutral 7-Me4APP submitted to a temperature-jump shows no relaxation phenomenon. Moreover, the UV spectrum of neutral 7-Me4APP is not significantly modified when recorded in water or in $CHCl_3$ and the IR spectrum recorded in $CHCl_3$ (Figure 4) shows two absorption bands readily assigned to $NH_2$ stretching [3528 (antisym) and 3414 $cm^{-1}$ (sym)].[28]

We shall thus admit that 7-Me4APP exists in water only as the amino tautomer. This observation parallels the conclusions for the structurally similar 3-substituted adenine.[29,30]

The high $pK_a$ value of 5-Me4APP and its close structural

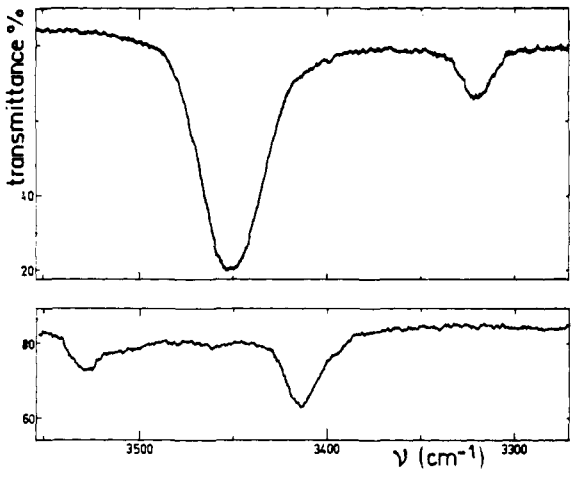
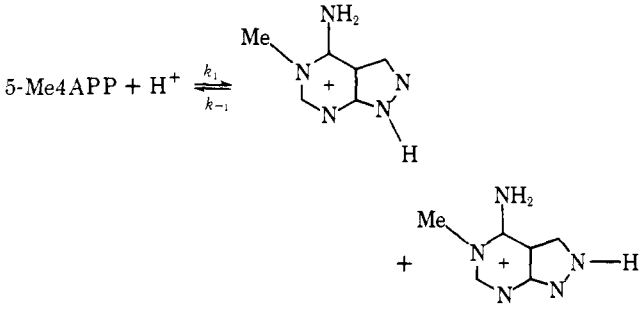



Figure 4. IR spectra in chloroform (optical length 2 cm): (upper) 5-Me4APP (saturated solution at 40 °C); (lower) 7-Me4APP (saturated solution at 40 °C).

resemblance to 1-methyladenine, in which the presence of the imino tautomer has been unambiguously established,[30] prompted us to examine whether or not 5-Me4APP might exist as an imine in aqueous solutions. The close examination of the $pK_a$ values of the various N-methylated derivatives provides a first approach to the answer.

Considering the following ionization equilibria and $pK_a$, it can be seen that the protonation of 5-Me4APP leads to two cations:




The equilibrium constant $K_5 = C_{2,5}/C_{1,5}$ has been previously estimated to be 0.3 so that

$$K_a = \frac{[(\text{amine}) + (\text{imine})][H^+]}{1.3C_{1,5}(\text{or } 4.3C_{2,5})} = 10^{-8.1}$$

and the value of

$$K_{\text{imine}} = \frac{[\text{imine}][H^+]}{C_{1,5}}$$

can be inferred from the $pK_a$ value of 1,5-dimethyl-4-iminopyrazolo[3,4-*d*]pyrimidine (1,5-diMe4IPP) (Table III). Hence, it is found that [amine]/[imine] = 3. Alternatively, the [amine]/[imine] ratio can be estimated from the $pK_a$ value of the *pure amino form* of 5-Me4APP by measuring the $pK_a$ of a nontautomerizable model compound. With this in mind we have synthesized 6,7-dihydro-8-methylimidazo[2,1-*f*]-pyrazolo[3,4-*d*]pyrimidine, which we henceforth refer to as 6,7-diH-8MePP.

This compound was preferred over 5-methyl-4-dimethylaminopyrazolo[3,4-*d*]pyrimidine not only because it is easily obtained, but mainly because the exocyclic N-$R_1R_2$ group remains in the plane of the pyrazolopyrimidine ring (as it is likely that $NH_2$ is in 5-Me4APP), whereas dialkylation of $NH_2$ would lead to a torsion of the C–N bond due to steric effects with the 5 substituent. Neutral 6,7-diH-8MePP will protonate according to the following scheme (Table III).

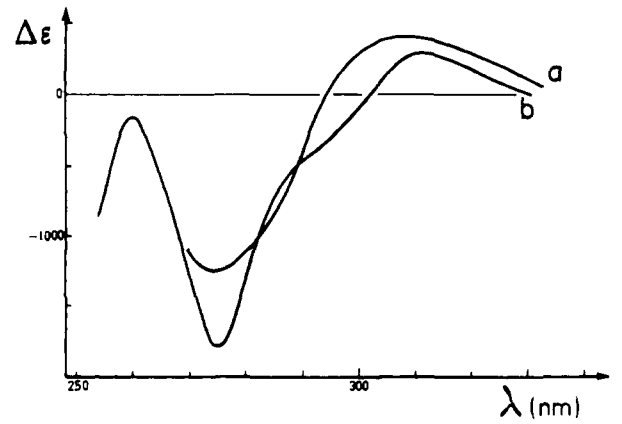
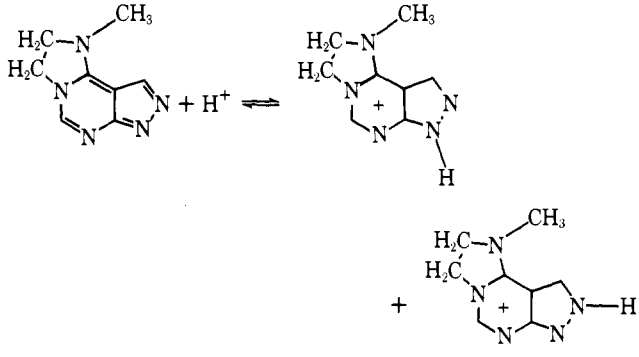



Figure 5. (a) Differential spectrum of 5-Me4APP recorded in pure THF and THF + 10% $H_2O$. (b) Differential spectrum of 1-5diMe4IPP and 6,7-diH-8MePP in water.

When as previously, $C_{2,5}/C_{1,5}$ is taken to be equal to 0.3, the [amine]/[imine] ratio in 5-Me4APP is readily estimated from



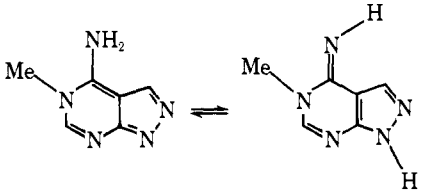

the above equilibrium together with the $pK_a$ of 1,5-diMe4IPP (Table III) and equals 4.5, a value which compares well, given the approximate nature of the methods, with that previously obtained. Given the expected marked difference in polarity of the imino and amino tautomers of 5-Me4APP, one can presume that the [amine]/[imine] ratio will be much affected by the change in the dielectric constant of the medium. This is actually observed. In chloroform, 5-Me4APP is present only as the less polar imino tautomer, as shown by the IR spectrum (Figure 4). The symmetric and antisymmetric stretchings of $NH_2$, expected to show respectively around 3530 and 3410 $cm^{-1}$ (when referring to the IR spectrum of 7-Me4APP), are totally absent, whereas the band at 3452 $cm^{-1}$ ($\epsilon \sim 200$) is unambiguously attributed to the vibration of the cyclic N–H bond, and the band at 3320 $cm^{-1}$ ($\epsilon \sim 20$) to the exocyclic imine.[30]

Moreover, isosbestic UV spectra of 5-Me4PP are observed in THF/water and dioxane/water mixtures for water concentrations ranging from 0 to 15%. The absorption spectrum in pure THF resembles that recorded in dioxane and chloroform where the imine tautomer is the major species, while increasing water concentration brings about the progressive formation of the amino tautomer. Furthermore, the variation with the wavelength of the absorption difference between two spectra recorded in two different $H_2O$/THF mixtures (differential spectrum) parallels the difference of the molecular extinction coefficients of 1,5-diMe4IPP and 6,7-diH-8MePP (Figure 5), thus establishing that the *imino* tautomer of 5-Me4APP is 5-Me-1-H4IPP and not 5-Me-2-H4IPP.

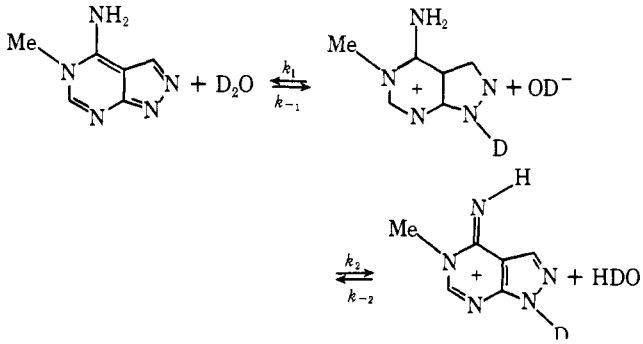
A direct estimate of the [amine]/[imine] ratio in aqueous 5-Me4APP is alternatively allowed by the fact that at $\lambda > 320$ nm only the imino form has an appreciable absorbance, as shown by comparsion of the UV spectra of an imino structure (either the model 1,5-diMe4IPP or 5-Me4APP in nonpolar

solvents) to the amino one (6,7-diH-8MePP). The [amine]/[imine] ratio is then found to be 7, when using the $\epsilon$ values of the fixed model compounds for the H-substituted tautomers. This value, obtained in a more straightforward manner, should be preferred over the one we inferred from the $pK_a$.

This favorable [amine]/[imine] ratio prompted us to investigate whether a chemical relaxation spectrum of *neutral* 5-Me4APP in $H_2O$ may be observed. Indeed, we recorded a short (<10 $\mu$s) pH-dependent relaxation time. In order to decrease the reaction rates so as to obtain more accurate kinetic data, the experiments are performed in $D_2O$. The variation of the relaxation time with pH follows the same pattern as in $H_2O$ and, when pD <9, depends on the initial 5-Me4APP concentration. The relaxation is attributed to the equilibrium[31]



where the interconversion of the two tautomers is catalyzed by the cation $C_{1,5}$ at pD < (9.5 (catalysis by $D^+$ is quite ineffective owing to the high pD range), and by $OD^-$ above pD 9.5 (Table I). However, the preponderant contribution to the reaction rates comes from the pD-independent term $k_0$ (Table I), which, since any significant nondissociative proton transfer is unlikely to occur, arises from the acidic catalysis by water:



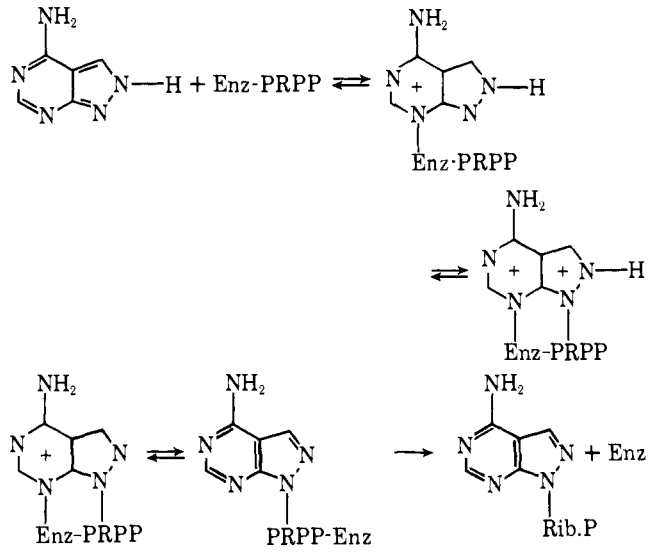
This term has been shown to be related to the $pK_a$ of the *less abundant* tautomer (eq 6′). Taking $k_1 = k_2 = 10^{10}\ M^{-1}\ s^{-1}$, in the preceding scheme, and the ionic product of $D_2O$, $K_{D_2O} = 10^{-15.5}$ at 10 °C, then $pK_{imine}$ equals 9.8 in $D_2O$ at 10 °C; assuming an equilibrium enthalpy of 9 kcal $mol^{-1}$, it is equal to 9 in $H_2O$ at 20 °C. The comparison of this value to the $pK$ of 6,7-diH-8MePP leads to [amine]/[imine] = 10, a value satisfactorily consistent with our previous estimates.

## Conclusion

This study has underlined the close qualitative similarity of 4APP and adenine. Like adenine, 4APP exists in water mainly as a mixture and has very similar acid-base properties. Moreover, the very small proportion of 4APP in water in the rare 1-H-5-H4IPP form and that of the N(1)-N(9) imino tautomer of adenine evaluated from the $pK_a$ of 9-methyladenine and 1,9-dimethyladenine are both roughly $2 \times 10^{-5}$. A marked similarity has also been observed between 5-Me4APP and 1-methyladenine, which are both present in water in measurable proportions of imino tautomer. Moreover, 5-Me4APP has proved to have the same hormonal property as 1-methyladenine in the induction of meiosis in starfish.[32]

However, some relevant quantitative differences between adenines and 4APPs must be mentioned. It is well established that although protonation of N(9) and N(7) substituted adenines at the N(3) and N(1) sites, respectively, is not excluded (despite the lack of any conclusive evidence), the predominant cationic species are N(1)-N(9) and N(7)-N(3)[12] while in 4APP the protonation of N(2)-substituted derivatives occurs to the same extent at N(7) and N(5), and N(1)-substituted derivatives lead to the major N(1)-N(7) cationic form. This means that *if the interaction* of adenine (or 4APP) with adenine phosphoribosyltransferase involving the N(3) and N(7) sites of adenine (N(7) and N(2) sites of 4APP as homologues of the sites of adenine) actually occurs, as postulated by Gadd and Henderson,[1] the "active" form of adenine capable of fixation to the enzymatic protein would be N(7)-H adenine (18% of the total aqueous adenine) with N(3) being the electron-rich site, or N(3)-H (0.5%) (N(7) being the basic site); parallelly, for 4APP it would be 7-H4APP (0.1%) or 2-H4APP (10%) which would combine either through N(7) or N)3) as these sites have similar basicities.

Scheme I



Tentatively the reaction of Scheme I for 4APP ribosylation is proposed where the formation of the dication would be the rate-determining step.

As concerns the rare imino tautomers, it appears that iminoadenine is somewhat more basic ($pK_a$ = 9.1 for the model compound) than 4IPP ($pK_a$(1,5diMe) = 8.6) so that at physiological pH the proportion of neutral 4IPP would be only *three times larger* than that of iminoadenine. This rules out the toxicity of 4APP toward cells arising from mispairing and subsequent fraudulent DNA formation, since 4APP appears to be as lowly mutagenic as adenine. Moreover, it has been established that allopurinol, which is *isoelectronic* to 4IPP, is not included in the DNA strand,[33] even at concentrations for larger than the actual concentration of 4IPP present in aqueous 4APP for clinical uses. As a consequence, the properties, of 4APP as adenine antimetabolite must somehow be related only to its abundant tautomers, 1-H- and 2-H4APP.

**Acknowledgments.** This work has been carried out with financial support from CNRS (ATP 1962). We wish to thank the Ciba-Geigy Corp. for its gift of substituted 4APP.

## References and Notes

(1) R. E. A. Gadd and J. F. Henderson, *Can. J. Biochem.*, **48**, 295 (1970).
(2) T. A. Krenitzky, S. M. Nell, G. B. Elion, and G. H. Hitchings, *J. Biol. Chem.*, **244**, 4779 (1969).
(3) K. Iwamoto and D. W. Martin, Jr., *Biochem. Pharmacol.*, **23**, 3199 (1974), and references cited therein.
(4) B. M. Lynch, R. K. Robins, and C. C. Cheng, *J. Chem. Soc.*, 2973 (1958).
(5) A correlation between the individual basicity, as determined *theoretically*, of the various nitrogen atoms in pyrazolopyrimidines and the antitumor

activity has been proposed by A. Pullman, B. Pullman, and T. Nakajima, *Bull. Soc. Chim. Fr.*, 590 (1959).
(6) G. P. Ceasar and J. J. Greene, *J. Med. Chem.*, **17**, 1122 (1974).
(7) J. F. Henderson and I. G. Junga, *Cancer Res.*, **21**, 118 (1961).
(8) R. J. Pugmire and D. M. Grant, *J. Am. Chem. Soc.*, **93**, 1880 (1971).
(9) R. J. Pugmire, D. M. Grant, L. B. Townsend, and R. K. Robins, *J. Am. Chem. Soc.*, **95**, 2791 (1973).
(10) M. T. Chenon, R. J. Pugmire, D. M. Grant, R. P. Panzica, and L. B. Townsend, *J. Heterocycl. Chem.*, **10**, 431 (1973).
(11) M. T. Chenon, R. P. Panzica, J. C. Smith, R. J. Pugmire, D. M. Grant, and L. B. Townsend, *J. Am. Chem. Soc.*, **98**, 4736 (1976).
(12) M. Dreyfus, G. Dodin, O. Bensaude, and J. E. Dubois, *J. Am. Chem. Soc.*, **97**, 2369 (1975).
(13) M. Dreyfus, O. Bensaude, G. Dodin, and J. E. Dubois, *J. Am. Chem. Soc.*, **98**, 6338 (1976).
(14) O. Bensaude, M. Dreyfus, G. Dodin, and J. E. Dubois, *J. Am. Chem. Soc.*, **99**, 4438 (1977).
(15) C. C. Cheng and R. K. Robins, *J. Org. Chem.*, **21**, 1240 (1956).
(16) E. C. Taylor and P. K. Loefler, *J. Am. Chem. Soc.*, **82**, 3147 (1960).
(17) P. Schmidt, K. Eichenberger, M. Wilhelm, and J. Druey, *Helv. Chim. Acta*, **42**, 763 (1959).
(18) T. P. Johnston, A. L. Fikes, and J. A. Montgomery, *J. Org. Chem.*, **27**, 973 (1962).
(19) M. J. Cook, A. R. Katritzky, P. Linda, and R. D. Tack, *J. Chem. Soc., Perkin Trans. 2*, 1295 (1972).
(20) R. G. Bates, "Determination of pH", Wiley-Interscience, New York, N.Y., 1973, p 375.
(21) The same value of the enthalpy was obtained from the variation of the UV spectrum of 4APP with temperature. This is made possible by the absence of any extinction coefficient change with temperature (demonstrated by the absence of a fast relaxation phenomenon accompanying the chemical relaxation) at the wavelength of observation ($\lambda$ = 305 nm), plus the fact that at this wavelength only the N(2)-H structure accounts for the overall absorbance of aqueous 4APP.
(22) When thermodynamically favorable, the protonation or $OH^-$ fixation occurs at the *same* rate (i.e., that of $H^+$ or $OH^-$ diffusion) at the various basic or acidic sites (M. Eigen, *Angew. Chem., Int. Ed. Engl.*, **3**, 1 (1964)). This hypothesis is considered valid for all the kinetic studies presented in this work. Moreover, in order not to multiply the number of kinetic symbols, the same symbols ($k_1$, $k_{-1}$, $k_2$, $k_{-2}$) will be used for *acid* or *base* catalysis when not ambiguous.
(23) The protonation of the imidazole ring of adenine occurs at fairly low pH values as shown by the absence of any autocatalytic pathway for the tautomeric interconversion.[12] Assuming that the *difference* of basicity between pyrazole and imidazole rings in *4APP and adenine*, respectively, is similar to the difference of basicity between pyrazole ($pK_a$ = 2.47) and imidazole ($pK_a$ = 6.95) leads to the reasonable conclusion that the common cation of 4APP would have a p*K* of formation around zero.
(24) R. A. Earl, R. J. Pugmire, G. R. Revankar, and L. B. Townsend, *J. Org. Chem.*, **40**, 1822 (1975).
(25) A very rough estimate of the proportion, $\alpha$, of 7-H-1-*i*-Pr4APP cation is obtained by assuming that, in the absence of this tautomeric cation, the downfield shift at $C_{7a}$ should be equal to the one at $C_3$ (−3.4 ppm) and that protonation at $N_7$ would produce a shift of 8.5 ppm at $C_{7a}$. This gives $-3.4(1 + \alpha) + 8.5\alpha = -1.9$; hence $\alpha \simeq 10\%$.
(26) For difficulties related to the synthesis of the nontautomerizable model compounds of the various H tautomers, the attribution of the relaxation spectrum cannot be achieved by the usual comparison of the relaxation amplitude with the differential spectrum. The assignments will be tentatively made on the basis of other kinds of evidence provided by NMR or UV spectroscopy, as well as by the consistency of the hypothesis with the subsequent observations and conclusions.
(27) The precautions that must be taken when estimating the tautomeric equilibrium constants from the p*K*s of fixed derivatives have been reviewed by J. Elguero, C. Marzin, A. R. Katritzky, and P. Linda, *Adv. Heterocycl. Chem., Suppl. 1*, 15–29 (1976).
(28) (a) D. J. Brown and S. F. Mason, *J. Chem. Soc.*, 682 (1957); (b) S. F. Mason, *ibid.*, 1281 (1959); (c) *ibid.*, 3619 (1958); (d) J. J. Elliot and S. F. Mason, *ibid.*, 1275 (1959).
(29) B. C. Pal and C. A. Horton, *J. Chem. Soc.*, 400 (1964).
(30) M. Dreyfus, G. Dodin, O. Bensaude, and J. E. Dubois, *J. Am. Chem. Soc.*, **99**, 7027 (1977).
(31) The existence of an equilibrium between the imino structures 5-Me-1-H4IPP and 5-Me-2-H4IPP is far from being unlikely. Preliminary work carried out in this laboratory on 4-hydroxypyrazolo[3,4-*d*]pyrimidine (allopuriono), which is *isoelectronic* to 5-MeIPP, has established the presence of 1-H and 2-H tautomers in aqueous solutions. However, the attribution of the relaxation spectrum in neutral 5-Me4APP to the equilibrium involving the imino structures may be ruled out on the basis of the following arguments: (a) the relaxation amplitude should be much weaker than that actually observed, (b) the observed acidic autocatalytic cannot be understood since the cation common to both imino tautomers would not form abundantly when the imino forms are protonated.
(32) (a) M. Dorée, personal communication; (b) M. Doree, P. Guerrier, and N. J. Leonard, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 1669 (1976).
(33) D. L. Nelson and G. B. Elion, *Biochem. Pharmacol.*, **24**, 1235 (1975).

# Five-Membered-Ring Hydrogen Rearrangement in Mass Spectral Fragmentations. Another Mechanism of $\gamma$ Cleavage

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**Abstract**: A new mechanism of $\gamma$ cleavage in the mass spectra of aliphatic carbonyl compounds was characterized by examining the spectra of deuterium-labeled butanoic acids. $\gamma$ cleavage occurs following five-membered-ring hydrogen transfer from the $\beta$ carbon to an oxygen and the shift of a hydrogen atom from the $\alpha$ to the $\beta$ carbon. This five-membered-ring hydrogen rearrangement is competitive with and occurs up to $^1/_3$ as frequently as the six-membered-ring hydrogen transfer which precedes the well known $\beta$-cleavage loss of an olefin.

The best known mass spectral rearrangement involves transfer of a $\gamma$ hydrogen to a carbonyl oxygen in conjunction with olefin loss by $\beta$ cleavage.[2-4] Much evidence[3,4] has indicated that six-membered-ring hydrogen transfer is highly specific, though competing hydrogen rearrangements by larger sized rings sometimes occur.[4] We here demonstrate that five-membered-ring hydrogen rearrangement followed by $\gamma$ cleavage[3] competes significantly with six-membered-ring hydrogen transfer-$\beta$ cleavage.

## Results

Table I gives the intensities of the ions formed by $\gamma$ cleavage and $\gamma$ cleavage followed by the loss of a water molecule in the normal mass spectra of several deuterium-labeled butanoic acids and the intensities of the ions of the corresponding compositions in the spectra of 2-ethylbutanoic acid and 2-ethylbutanoic acid-*O*-$d_1$. The only detected metastable decompositions of the $C_4H_8O_2^{+\cdot}$ ions were the losses of methyl