Tautomerism of Formycin. Mechanism of Interconversion

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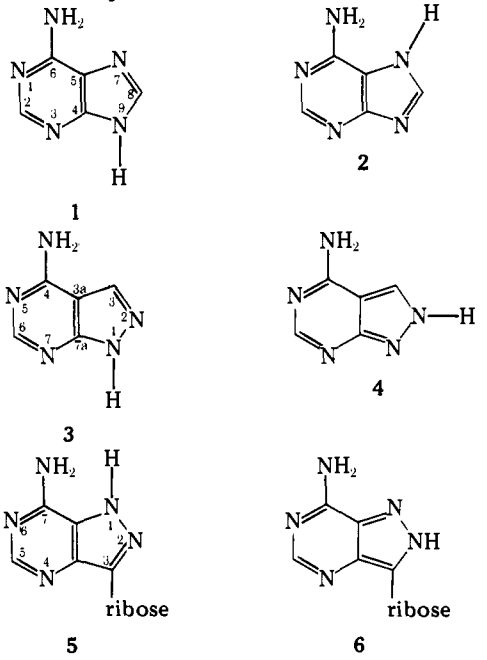
Abstract: The tautomeric equilibrium between 1*H*- and 2*H*-formycin (7-amino-3β-D-ribofuranosyl-1*H*-pyrazolo[4,3-*d*]pyrimidine) has a constant K_T = N(2)H/N(1)H = 0.2 and an enthalpy estimated as 1 kcal mol^{-1}. The tautomeric interconversion is catalyzed by H^+ (k_{H^+} = 3 × 10^9 M^{-1} s^{-1}) and by OH^- (k_{OH^-} = 5 × 10^9 M^{-1} s^{-1}). No other catalytic pathway such as water catalysis or tautomerization via tautomeric cations contributes significantly to the interconversion. Protonation of formycin does not occur significantly on the pyrazole ring.

The relative basicities of the various nitrogen sites of adenine and adenine analogue 4-aminopyrazolo[3,4-*d*]pyrimidine (4-APP) is expected to be a relevant property as regards the interaction with enzymes (through orientation of hydrogen bonding) and the reaction with methylating agents.

It has been inferred from UV and NMR studies of the protonation of its alkylated derivatives that the basic site of 9*H*-adenine (**1**) (the most abundant tautomer) is the N(1) atom, whereas the most basic nitrogen atom of 7*H*-adenine (**2**) is N(3).[1,2] In 4-APP N(5) is the most basic site of the abundant tautomer N(1)H (**3**) and in the N(3)H (**4**) tautomer N(5) and N(7) have comparable basicities.[3] A more direct insight (since it involves the unsubstituted derivatives themselves) into cation structure (and hence basicity) is provided by the analysis of proton-exchange kinetics in these tautomerizable systems. It is well established that the tautomerization of heterocyclic compounds proceeds, among other reaction pathways, via an acid-catalyzed step. The acid catalysis involves an intermediate cation obtained from both tautomers by single proton transfer (i.e., the cation is *common* to both tautomers).[1,3-5] It has been shown that, when the intermediate common cation is the main species resulting from protonation of either tautomer, acid catalysis by this entity (autocatalysis) is observed.[4,5] The absence of autocatalytic contribution (even when the pK_a is high enough to allow sufficient proportions of cationic species) can thus be taken as a convenient criterion for asserting that the common cation is not the predominant protonated form.[1,3] On this ground, it has been postulated that protonation of the imidazole moiety of adenine and of the pyrazole moiety of 4-APP occurs only to a low extent.



1 **2** **3** **4** **5** **6**

4-Aminopyrazolo[4,3-*d*]pyrimidines (**5** and **6**) (an interesting class of adenine antimetabolites), which have been shown to exist as a tautomeric mixture,[6] have received so far only little attention as regards the location of protonation sites,[7] and the partial kinetic results that have appeared on formycin tautomerism mechanism do not allow significant conclusions on this matter.[8] We wish to report a more complete kinetic study on this latter tautomeric system which was undertaken to assign the basic sites of formycin from kinetic evidence with the hope that this might contribute to the understanding of the antibiotic properties of this compound.

Experimental Section

The conditions of the temperature-jump experiments and pH measurements were as described previously.[1,3-5] Formycin (gift from Dr. H. Buc, Institut Pasteur) was found homogeneous on TLC.

Results and Discussion

After a temperature jump, a relaxation phenomenon is observed (the amplitude at 313 nm is 94 OD units/mol). It is readily attributed to the N(1)H ⇄ N(2)H tautomeric equilibrium. The enthalpy of the equilibrium, ΔH = 1 ± 0.1 kcal, is evaluated from the variation of the relaxation amplitude with initial temperature, at constant temperature-jump magnitude.[1,3] The equilibrium constant can thus be roughly estimated[1] and is found to be 0.2 (±0.1) when $\epsilon^{313}_{N(1)H} - \epsilon^{313}_{N(2)H}$ is taken equal to 6000 at 313 nm (as inferred from the absorbances at N^1-methylformycin and N^2-methylformycin).[6,9]

The exchange rate is pH dependent, and does not depend significantly on formycin concentration in the 3 × 10^{-4} to 5 × 10^{-5} M concentration range (Figure 1). The kinetics is readily understood in terms of acid/base catalysis which involves the intermediate ionized species *common* to both neutral tautomers.

The minimum value of the reciprocal relaxation time (10^3 s^{-1} at pH 7) is satisfactorily accounted for by H^+ and OH^- catalysis only, thus excluding significant contribution from other tautomerization processes such as direct proton transfer from N(1) to N(2) and/or water catalysis. The former process requires a particular geometry of the exchanging sites[10] and was never detected in tautomerism involving the pyrazole ring.[3,11] Prior calculation from the pK values of formycin would have shown that noticeable participation by the latter process can be rejected.[3-5]

Base Catalysis. The reaction proceeds through an intermediate common anion (Scheme I). Applying the steady-state approximation to the intermediate anion leads to $\tau_b^{-1} = k_{OH^-}[OH^-]$. The value k_{OH^-} = 5 × 10^9 (0.2) M^{-1} s^{-1}, though consistent with that expected from a rate encounter controlled process, is somewhat lower than that observed in other heterocyclic systems, probably because the bulky sugar moiety of formycin reduces the encounter rate.

The absence of detectable concentration effects (autocatalysis) on the relaxation time would arise from a low encounter

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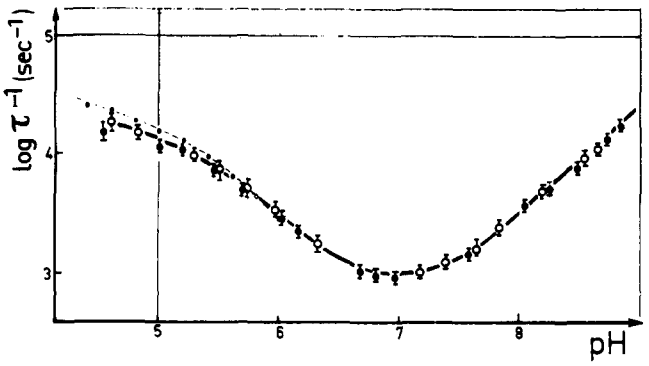


Figure 1. Log τ^{-1} as a function of pH$^+$: (○), $C_{formycin} = 5.4 \times 10^{-4}$ M; ●, $C_{formycin} = 2.36 \times 10^{-4}$ M. Theoretical plot according to eq 1 in Appendix II ($C^0_{formycin} = 10^{-4}$, p$K_1 = 4$, p$K_2 = 5.7$, p$K_F = 4.8$, $k_{H^+} = 3 \times 10^9$ M^{-1} s^{-1}).

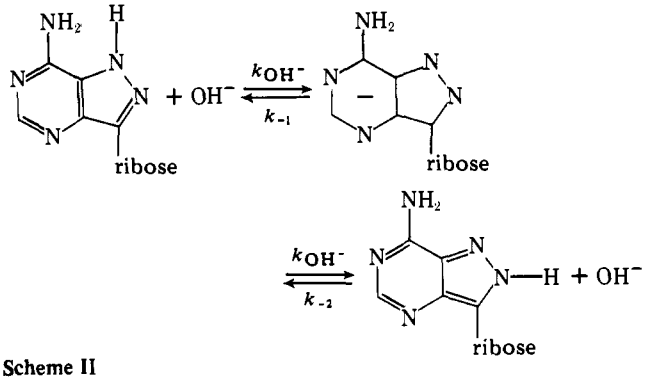
rate between neutral and charged species or/and from a weak concentration in the common ionized species. Autocatalytic contributions have been observed at basic pH compounds with similar pK values (proton loss), such as adenine and isocytosine.[1,10] The origin of autocatalysis inefficiency in the basic range must hence be sought in the molecule/ion encounter rate, which is likely to be low because of the ribose substituent[1,3-5]

Acid Catalysis. At acidic pHs, protonation is likely to occur preferentially at the pyrimidine ring, as reasonably inferred from the protonation patterns of adenine[1] and 4-APP,[3] and hence the common cation is not the abundant charged species. This, together with the low value for the molecule/ion rate constant, is an additional reason why autocatalysis is not observed at low pHs.

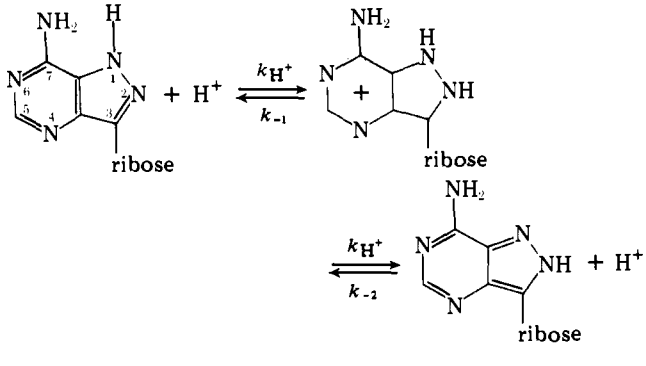
The pathway in Scheme II predicts $\tau_a^{-1} = k_{H^+}[H^+]^1$ and successfully accounts for the acid catalysis in most tautomeric systems involving heteroatom (with the exception of 4APP, where the pH/rate profile departs slightly from linearity).[1,3-5]

For formycin, however, the plot of log τ^{-1} vs. pH in the acidic range has a slope (markedly less than unity) which varies with pH (Figure 1). This shows that the simple model (Scheme II) is not fully satisfactory and that a more elaborate one

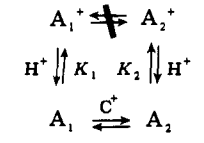
Scheme I



Scheme II



Scheme III[a]



[a] A_1 and A_2 stand for the N(1)H and N(2)H tautomers, respectively.

should take into account the presence of appreciable amounts of cationic species other than the common cation, and specific to each neutral tautomer (A_1^+, A_2^+) (Scheme III). A priori calculation shows that N(1)H-N(2)H neutral formycin tautomerization through the exchange of the tautomeric cations A_1^+ and A_2^+ is negligible (see Appendix I).

When it is assumed that equilibration between neutral species A_1 and A_2 and their specific cations A_1^+ and A_2^+ occurs much faster than tautomerization, it is shown that the relaxation time may be expressed as (see Appendix II)

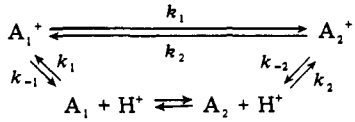
$$\tau^{-1} = \frac{k_{H^+}[H^+]}{1 + K_T}\left[K_T + \frac{1 + \frac{[H^+]}{K_1}\frac{[K_F + C_0]}{[K_F + C_0 + [H^+]]}}{1 + \frac{[H^+]}{K_2}\frac{[K_F + C_0]}{[K_F + C_0 + [H^+]]}}\right] \quad (1)$$

where K_1 and K_2 are the ionization constants of respectively 1*H*- and 2*H*-formycin to yield their *specific* cations. From a practical standpoint fitting the experimental data at acidic pHs with eq 1 is unrealistic. However, when reasonable values of the constants are introduced into eq 1, a set of pH/rate profiles may be computed for various values of K_1 and K_2. A typical calculation, when p$K_{formycin} = 5$,[12] $k_{H^+} = 4 \times 10^9$ M^{-1} s^{-1} (as calculated from the experimental data in the narrow pH range between 6 and 6.5), $K_T = 0.2$, p$K_1 = 4$, p$K_2 = 5.7$, depicts a slight dependence on the analytical formycin concentration at low pH and fits reasonably well the experimental pH/rate plot (Figure 1). The curvature at low pH may thus be considered as a hint for (a) $K_1 > K_2$ and (b) a large difference between the basicities of the neutral tautomers.

In conclusion, it may be stated, from the absence of acid autocatalysis, that formycin does not protonate significantly at the imidazole ring. This hypothesis is also supported by the fact that *protonated* formycin is alkylated at the imidazole moiety, the basic nitrogen atom on the pyrimidine ring being already substituted by a proton.[13] Moreover, the N(2)H tautomer is expected to be markedly more basic than the N(1)H form (as in 4-APP). One would expect thus that, although N(2)H is the less abundant form, the overall properties of formycin with respect to electrophilic addition (such as methylation or H-bond orientation) would be those of this latter tautomer.

Appendix I

It has been shown that cation tautomerization is base and acid catalyzed:[3]



where A_1 and A_2 stand for N(1)H and N(2)H, respectively. The expression of the relaxation time $(\tau)^{-1}$ for a pathway involving cation exchange is

$$(\tau)^{-1} = \left[\frac{k_1 k_1}{k_{-1} + k_1[H^+]} + \frac{k_2 k_2}{k_{-2} + k_2[H^+]}\right][H^+] \quad (2)$$

where k_1 and k_2 are composite rate constants, or roughly

$$(\tau)^{-1} = \frac{k_2 k_2[H^+]}{k_{-2} + k_2[H^+]}$$

since the second term of the second member in eq 2 is the rate constant of the less abundant tautomer N(2)H formycin, which is five times larger than that of N(1)H formycin (K_T = 0.2).

$$(\tau)^{-1} = \frac{k_2[H^+]}{K_2 + [H^+]} \quad (3)$$

where K_2 is the formation equilibrium constant of A_2^+.

Rate constant k_2 is simply related to the relaxation time of $A_1^+ \rightleftarrows A_2^+$ tautomeric equilibrium, $(\tau^{-1})_C$:

$$(\tau^{-1})_C = k_1 + k_2 = k_2[1 + K_{A_1^+/A_2^+}]$$

Tautomerization of the cations proceeds via the classical acid- and base-catalyzed mechanism and the expression of the relaxation times:

$$(\tau^{-1})_C = k_{H^+}[H^+] + k_{OH^-}[OH^-] + k_{autocat}[A] + k_0 \quad (4)$$

where [A] is the concentration of the neutral tautomer obtained from deprotonation of both cations.

Thus, the general expression for neutral tautomers exchange relaxation time is

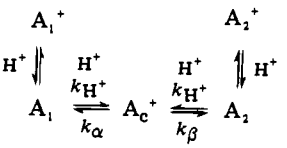
$$(\tau)^{-1} = \frac{1}{(K_2 + [H^+])(1 + K_{A_1^+/A_2^+})} \times [k_{H^+}[H^+] + k_{OH^-}[OH^-] + k_{autocat}[A] + k_0][H^+] \quad (5)$$

Even for high $[H^+]$, τ^{-1} will have a degree one in $[H^+]$.

The fact that the logarithm of the experimental relaxation time vs. pH displays a slope less than unity shows that contribution of this pathway is negligible. This arises from low values of the k_0 term in eq 4 (which is related to catalysis by water and to the pK of the less abundant cationic tautomer[3-5]) and/or high values of the tautomeric cation equilibrium constant.[3]

Appendix II. Obtention of (1)

A_c^+ is in a steady state and, in comparison to tautomerization, *ionizations of A_1 and A_2 are fast*.



After a temperature jump, one obtains

$$\delta[A_1] + \delta[A_2] + \delta[A_1^+] + \delta[A_2^+] = 0 \quad (6)$$

$$\delta[A_1^+] + \delta[A_2^+] + \delta[H^+] = 0 \quad (7)$$

Using $K_1 = [A_1][H^+]/[A_1^+]$ together with $K_2 = [A_2]\cdot[H^+]/[A_2^+]$ gives

$$\delta[A_1]/[A_1] + \delta[H^+]/[H^+] = \delta[A_1^+]/[A_1^+] \quad (8)$$

$$\delta[A_2]/[A_2] + \delta[H^+]/[H^+] = \delta[A_2^+]/[A_2^+] \quad (9)$$

$$\delta[A_1^+] = [A_1^+]\left[\frac{\delta[A_1]}{[A_1]} + \frac{\delta[H^+]}{[H^+]}\right]$$

$$\delta[A_2^+] = [A_2^+]\left[\frac{\delta[A_2]}{[A_2]} + \frac{\delta[H^+]}{[H^+]}\right]$$

$$\delta[A_1^+] + \delta[A_2^+] = \frac{\delta[H^+]}{[H^+]}[[A_1^+] + [A_2^+]] + [A_1^+]\frac{\delta[A_1]}{\delta[A_1]} + [A_2^+]\frac{\delta[A_2]}{[A_2]} = -\delta[H^+]$$

$$\delta[H^+]\left[1 + \frac{1}{K_F}[[A_1] + [A_2]]\right] + \frac{[H^+]}{K_1}\delta[A_1] + \frac{[H^+]}{K_2}\delta[A_2] = 0$$

where K_1, K_2, and K_F are the ionization constants of A_1, A_2, and overall formycin, A_T is the overall concentration of neutral formycin *at a given pH*, and C^0 is the analytical concentration of formycin. Furthermore

$$\delta[A_1]\left[\frac{K_F + A_T}{K_F} + \frac{[H^+]}{K_1}\right] + \delta[A_2]\left[\frac{K_F + A_T}{K_F} + \frac{[H^+]}{K_2}\right] = 0$$

$$\delta[A_1]\left[1 + \frac{[H^+]}{K_1}\frac{K_F}{K_F + A_T}\right] + \delta[A_2]\left[1 + \frac{[H^+]}{K_2}\frac{K_F}{K_F + A_T}\right] = 0$$

$$\delta[A_2] = -\delta[A_1]\frac{1 + \frac{[H^+]}{K_1}\frac{K_F}{K_F + A_T}}{1 + \frac{[H^+]}{K_2}\frac{K_F}{K_F + A_T}}$$

with $A_T = C^0K_F/([H^+] + K_F)$

$$\delta[A_2] = -\delta[A_1]\frac{1 + \frac{[H^+]}{K_1}\frac{K_F + [H^+]}{K_F + [H^+] + C_0}}{1 + \frac{[H^+]}{K_2}\frac{K_F + [H^+]}{K_F + [H^+] + C_0}}$$

Applying the steady-state approximation to A_c^+ leads to

$$\frac{d\delta[A_1]}{dt} = -\frac{k_{H^-}[H^+]}{k_\alpha + k_\beta} \times \left[k_\beta + k_\alpha\frac{1 + \frac{[H^+]}{K_1}\frac{K_F + [H^+]}{K_F + [H^+] + C^0}}{1 + \frac{[H^+]}{K_2}\frac{K_F + [H^+]}{K_F + [H^+] + C^0}}\right] + \text{const}$$

$k_\beta/k_\alpha = K_T$, the tautomeric constant. Hence

$$\tau^{-1} = \frac{k_{H^+}[H^+]}{1 + K_T}\left[K_T + \frac{1 + \frac{[H^+]}{K_1}\frac{K_F + [H^+]}{K_F + [H^+] + C^0}}{1 + \frac{H^+}{K_2}\frac{K_F + [H^+]}{K_F + [H^+] + C^0}}\right] \quad (1)$$

References and Notes

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