References and Notes

(1) Part 10: I. Szele and H. Zollinger, *J. Am. Chem. Soc.*, preceding paper in this issue.
(2) Presented in part at the 26th IUPAC Congress, Tokyo, Sept 4–10, 1977.
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(4) V. J. Shiner, Jr., W. Dowd, R. D. Fisher, S. R. Hartshorn, M. A. Kessick, L. Milakofsky, and M. W. Rapp, *J. Am. Chem. Soc.*, **91**, 4838 (1969).
(5) R. G. Bergstrom, R. G. M. Landells, G. H. Wahl, Jr., and H. Zollinger, *J. Am. Chem. Soc.*, **98**, 3301 (1976).
(6) G. A. Swan and P. Kelly, *J. Chem. Soc.*, 416 (1954).
(7) R. Wiebe, V. L. Gaddy, and C. Heins, Jr., *Ind. Eng. Chem.*, **24**, 823 (1932).
(8) E. S. Lewis and R. E. Holliday, *J. Am. Chem. Soc.*, **88**, 5043 (1966).
(9) D. M. Maharajh and J. Walkley, *Nature (London)*, **236**, 165 (1972).
(10) K. R. Brower, *J. Am. Chem. Soc.*, **82**, 4535 (1960).
(11) J. Miller, "Aromatic Nucleophilic Substitution", Elsevier, Amsterdam, 1968, p 29 ff, and references cited therein.
(12) H. Zollinger, *Acc. Chem. Res.*, **6**, 335 (1973).
(13) E. S. Lewis and W. H. Hinds, *J. Am. Chem. Soc.*, **74**, 304 (1952); E. S. Lewis, L. D. Hartung, and B. M. McKay, *ibid.*, **91**, 419 (1969).
(14) P. Burri and H. Zollinger, *Helv. Chim. Acta*, **56**, 2204 (1973).
(15) R. G. Bergstrom, G. H. Wahl, Jr., and H. Zollinger, *Tetrahedron Lett.*, 2975 (1974).
(16) R. G. M. Landells, Ph.D. Thesis, University of Edinburgh, 1976.
(17) The error in k_r is the largest of all the values determined. This is due to the fact that k_r is calculated from $P(\%)$ (via k_r/k_s) and k_s, and may thus accumulate errors involved in the determination of both these values.
(18) C. G. Swain, J. E. Sheats, and K. G. Harbison, *J. Am. Chem. Soc.*, **97**, 783 (1975).
(19) C. G. Swain, J. E. Sheats, D. G. Gorenstein, and K. G. Harbison, *J. Am. Chem. Soc.*, **97**, 791 (1975).
(20) C. G. Swain, J. E. Sheats, and K. G. Harbison, *J. Am. Chem. Soc.*, **97**, 796 (1975).
(21) H. G. Richey, Jr., and J. M. Richey in "Carbonium Ions", Vol. II, G. A. Olah and P. v. R. Schleyer, Ed., Wiley-Interscience, New York, N.Y., 1970, p 923.
(22) D = diazonium ion; X and Y = reaction intermediates; P = reaction products; Nu = any nucleophile present in solution (i.e., solvent, N_2, BF_4^-, etc.).
(23) In the computer fitting of the experimental data as well as in the F-test of one model against the other, individual measurements and not the mean values were used. This way, the relatively large errors in the rate measurements (Table VI) are taken into account, and it is shown that they do not interfere with the differentiation of the two models by the F-test.
(24) The actual rate of return from the first intermediate to the diazonium ion should be twice the rate of isotopic rearrangement, since, assuming two equivalent nitrogens, only half of the returns result in rearrangement. This implies that at high concentrations of SCN^-, provided that all the intermediate is trapped, rate increases of more than 40% are expected.
(25) A third, solvent-separated molecule–ion pair intermediate could also account for the exchange reaction, but all our results can be explained by two intermediates only.
(26) J. D. Dill, P. v. R. Schleyer, J. S. Binkley, R. Seeger, J. A. Pople, and E. Haselbach, *J. Am. Chem. Soc.*, **98**, 5428 (1976).
(27) J. D. Dill, P. v. R. Schleyer, and J. A. Pople, *J. Am. Chem. Soc.*, **99**, 1 (1977).
(28) A. Streitwieser, Jr., and A. Dafforn, *Tetrahedron Lett.*, 1435 (1976); L. R. Subramanian, M. Hanack, L. W. K. Chang, M. A. Imhoff, P. v. R. Schleyer, F. Effenberger, W. Kurtz, P. J. Stang, and T. E. Dueber, *J. Org. Chem.*, **41**, 4099 (1976).

Intramolecular Proton-Transfer Mechanism in the Uracil Monoanions and Derivatives. A Temperature-Jump Study

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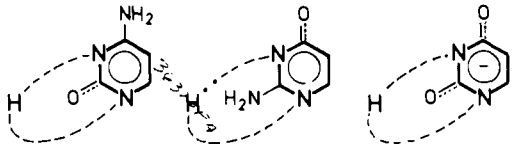
Abstract: The tautomeric interconversion of the monoanions of uracil and of 5-fluorouracil are shown by laser and joule-heated temperature-jump spectroscopy to proceed via a two-step mechanism involving the protonated or the deprotonated forms common to both tautomers. No contribution from a bifunctional nondissociative proton transfer mechanism involving water molecules is detected in the interconversion kinetics.

Introduction

Interconversion between the N(1)H and the N(3)H tautomers of the neutral pyrimidines, cytosine[2] (Cy) and isocytosine[3] (iCy), has been shown from temperature-jump relaxation experiments to proceed, in aqueous solutions, through intermediate protonation or deprotonation leading to a species common to both tautomers. This last species is then deprotonated or protonated yielding one of the tautomers ("D mechanism," dissociative).[4] In contrast, in the structurally closely related case of the uracil monoanion (U^-)[5] for which the D



mechanism is expected, it has been reported[6a] from dynamic NMR data that a concerted nondissociative proton transfer involving water molecules ("ND mechanism" nondissociative) would greatly contribute to the tautomeric interconversion rate.

Contribution of the ND mechanism to tautomeric interconversion has been shown by us[3] to be strongly related to the geometry of the tautomeric systems. It therefore seems that either the presence of a negative charge favors the ND mechanism, or the dynamic NMR technique gives information different from that obtained by relaxation spectrometry on aqueous solutions containing tautomeric molecules. Among the various systems studied[6] by the dynamic NMR technique, only the uracil monoanions absorb in the near UV, thus providing a unique opportunity to compare both methods.

Methods

Laser Temperature-Jump Experiments. Quantitative measurements on fast relaxation spectra (relaxation time smaller than 5 μs) were performed with the previously described laser temperature-jump apparatus.[7] For these measurements the detection bandwidth was limited to 5 MHz, in order to reduce

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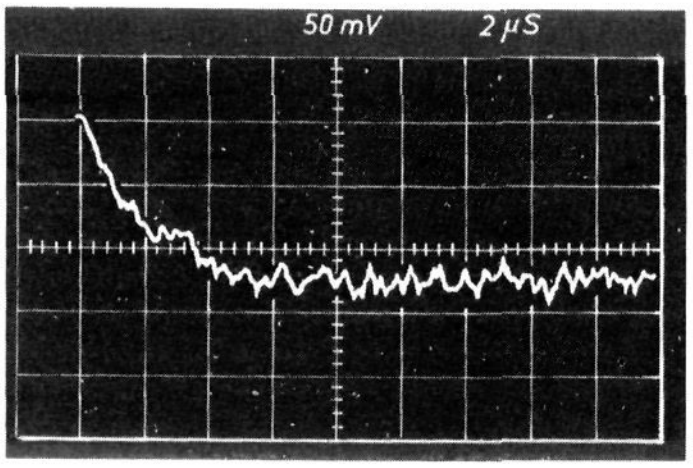


Figure 1. Laser temperature-jump relaxation spectrum of an aqueous uracil solution: pH 11.0; substrate concentration $c = 3.2 \times 10^{-3}$ M; optical path 0.6 mm; $t_i = 4$ °C and $t_f = 10 \pm 2$ °C; observation wavelength λ 293 nm. Vertical scale: 50 mV/major division. 60 mV represents an optical density (OD) change of 5×10^{-3} units. Horizontal scale: 2 μs/major division. The signal corresponds to an increase of the solution OD.

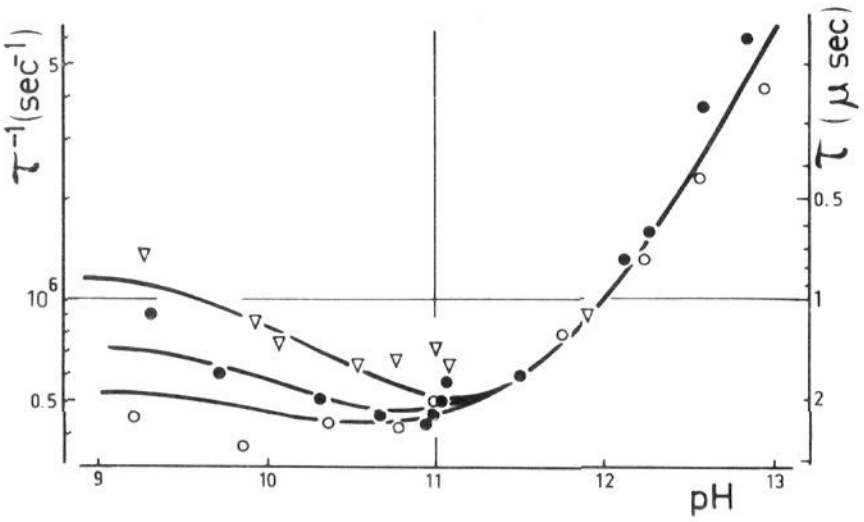


Figure 2. Laser temperature-jump study of the relaxation time as a function of pH for uracil monoanions: (—) best fit of the experimental data with eq 1. Substrate concentrations: ▽, 7.2×10^{-3} M; ●, 3.2×10^{-3} M; ○, 1.5×10^{-3} M; $t_i = 4$ °C and $t_f = 10 \pm 2$ °C; λ 293 nm.

the shot noise; since the spectrometer light source (a xenon 150-W arc lamp) was pulsed when firing the laser, there was a considerable improvement in the signal to noise ratio.[8] The Polaroid pictures (Figure 1) were digitized by means of a Matra analogic digital transducer system (S.T.R.A.N.D.) coupled with a PDP-8 processor (Digital Equipment Corp.). The relaxation times were computed by performing a least-squares regression. A kinetic run consisted of three to six relaxation spectra. The pH (measured with an EA 125 Metrohm combined electrode fitted on a circulating device) was adjusted by addition of aliquots of NaOH and HCl. Standard experiments were performed at 0.6-mm optical length with $t_i = 4$ °C and $t_f \simeq 10$ °C. The solutions were degassed prior to use. In order to avoid photochemical decomposition, illumination was cut down except when recording the relaxation curve. UV spectra of the solutions diluted in appropriate buffers before and after a kinetic run were identical within experimental error in every case, and the concentrations were determined from the optical densities.

Joule-Heated Temperature-Jump Experiments. Temperature-jump experiments were performed with the apparatus and circulating device described previously[9] together with the modifications introduced later.[2] Standard experimental conditions were initial temperature $t_i = 2$ °C and final temperature $t_f = 10$ °C. The use of concentrations from 10^{-5} to 10^{-3} M brought the interconversion relaxation time into the 10^{-4}–10^{-5} s range. A kinetic run consisted of at least three series of accumulations of three to nine relaxation spectra. The mean values for the relaxation time and for the corresponding pH were kept for further computations. The pH was continuously measured with a Radiometer type 64 pH meter,

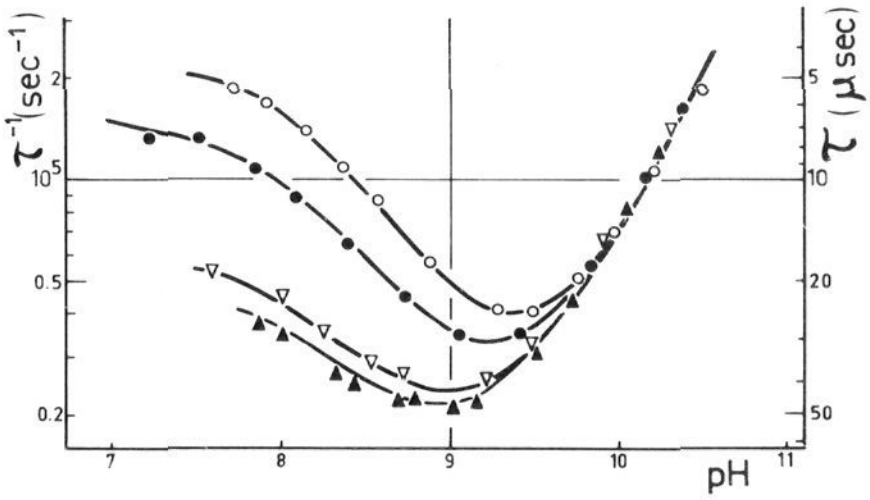


Figure 3. Joule temperature-jump study of the relaxation time as a function of pH for 5-fluorouracil monoanions: (—) best fit of the experimental data with eq 1. Substrate concentrations: ○, 1.6×10^{-3} M; ●, 0.96×10^{-3} M; ▽, 0.35×10^{-3} M; ▲, 0.28×10^{-3} M; $t_i = 2$ °C and $t_f = 10$ °C; λ 295 nm.

Table I. Thermodynamic Data Used in This Work[a]

	pK_1	pK_2	K_T
Uracil	9.74[12]	13.5[13] (25 °C)	0.885[14]
5-Fluorouracil	8.13[b]	12.6[c]	0.5[15]

[a] At 10 °C and $\mu = 0$ unless otherwise stated. [b] Measured by potentiometry. [c] Measured by spectrophotometry in NBS buffers.

equipped with a G202C Radiometer glass electrode and a NaCl reference electrode; the difference between its initial and final value never exceeded 0.05 pH units. Solutions for the kinetic runs were prepared by dissolving weighed amounts of compounds in 0.2 M solutions of $NaClO_4$, H_2O (Merck reagent grade) in distilled water. Both uracil and 5-fluorouracil (Aldrich) were recrystallized from water and their purity was checked by TLC.[10]

Results

When a solution of uracil monoanion (U^-) is subjected to a rapid temperature rise, the optical density at 293 nm increases exponentially with a time constant smaller than 3 μs. This is too fast to be studied by the conventional Joule-heating technique, unless high ionic strengths are used, which would make the conversion of pH into concentrations inaccurate. Therefore our laser temperature-jump apparatus (heating time 20 ns) was well adapted for this quantitative study. The relaxation time is pH dependent, maximizes around pH 10.5, and is shortened at lower pH by increasing substrate concentration (Figure 2). Thus, the relaxation time, τ, is fitted to the equation[11]

$$\tau^{-1} = k_0 + k_{OH^-}\overline{C}_{OH^-} + k_{UH_2}\overline{C}_{UH_2} \qquad (1a)$$

where $\overline{C}_i$ is the equilibrium concentration of species "i" at the final temperature, and is computed as described in the appendix of ref 9, using the thermodynamic data in Table I. A weighted least-squares regression program[3] is used, and the parameters of the best fit are given in Table II.

The relaxation amplitude increases above pH 9 and becomes roughly pH independent in the 11–12.5 pH range. However, it yields little information, owing to the low reproducibility of the temperature variations and the difficulty in observing very small signals at wavelengths below 280 nm (arising from the weakness of our light source at low wavelengths despite use of a pulsing mode[8]).

The optical density changes of the 5-fluorouracil monoanion (FU^-) aqueous solutions are much slower than for U^-, and can therefore be studied by conventional Joule-heating temperature-jump spectroscopy. The amplitudes of the relaxations

Table II. Kinetic Rate Constants According to Equations 1a and 1b[a]

	$10^{-4}k_0$, s^{-1}	$10^{-10}k_0(\bar{K}_1/\bar{K}_W)$, $M^{-1}\,s^{-1}$	$10^{-9}k_{OH^-}$, $M^{-1}\,s^{-1}$	$10^{-8}k_{UH_2}$, $M^{-1}\,s^{-1}$	ρ	N	10^3 concn range, M
Uracil	36.5 ± 3	2.25 ± 0.2	0.39 ± 0.04	1.13 ± 0.2	0.9988	32	1.5-7.2
5-Fluorouracil	0.85 ± 0.044	2.15 ± 0.1	3.9 ± 0.1	1.5 ± 0.025	0.999 925	44	0.28-1.6

[a] Results refer to experiments in aqueous solutions at t_f = 10 °C. Standard errors are given. ρ is the correlation coefficient defined as in ref 3. N is the number of kinetic runs used in the correlation.

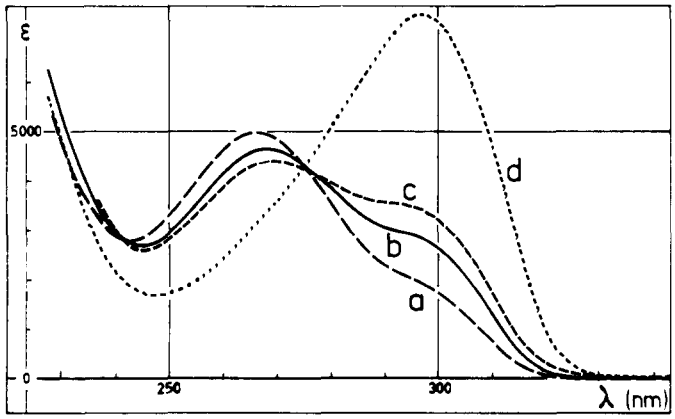


Figure 4. UV spectra of 5-fluorouracil monoanions in (a) 5 M $NaClO_4$, pH 10 NBS buffer aqueous solution at 21 °C; (b) pH 10 NBS buffer (ionic strength μ = 0.1 M) at 21 °C; (c) pH 10 NBS buffer at 53 °C; (d) 2 × 10^{-3} M NaOH solution in methanol at 20 °C.

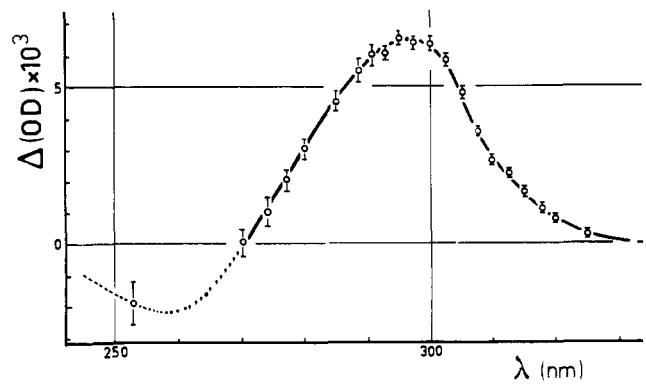


Figure 5. Wavelength dependence of the relaxation amplitude of 5-fluorouracil monoanion. Substrate concentration $c = 0.8 \times 10^{-4}$ M; t_i = 2 °C and t_f = 10 °C; optical path 7 mm.

are then reliable and help in the attribution of the observed phenomena. The relaxation time variations with pH and substrate concentration (Figure 3) are fitted to the equation[11]

$$\tau^{-1} = k_0 + k_{OH^-}\bar{C}_{OH^-} + k_{FUH_2}\bar{C}_{FUH_2} \qquad (1b)$$

The results are given in Table II.

Discussion

Attribution of the Observed Relaxation Spectra. A number of workers have established that the spectra of the monoanionic forms of uracil,[14] thymine,[15] 5-halouracils,[15,16] and 4-thiouracil[17] are composite spectra of the individual N(1)H and N(3)H monoanions; the proportions of these, and hence the UV and IR spectra, are strongly altered by ionic strength,[17,18] solvent changes,[15,18] and *temperature*.[15] The close similarity in the behavior of these compounds prompted us to back up our study by an investigation of the 5-fluorouracil monoanion (FU^-) relaxation which can be unambiguously attributed to the tautomeric interconversion on the following basis:[3] (a) The wavelength dependence of the optical density changes associated with the medium effects (Figure 4) agree with the wavelength dependence of the relaxation amplitude (Figure 5). (b) The relaxation amplitude is proportional to the sum of the total tautomer concentrations, i.e., the total monoanion concentration (C_{FU^-}) computed from knowledge of the pH. (c) The relaxation amplitude corresponds to the temperature effects on the UV spectrum (Figure 4). The relaxation amplitude of uracil monoanion (U^-)[5] also satisfies these criteria within the experimental error and as long as the measurements are feasible. Therefore relaxation signals following the fast heating of monoanionic uracil solutions can also be attributed to the tautomeric interconversion.

Nevertheless, attribution of the observed phenomenon to the reaction

$$UH_2 + OH^- \underset{k_f}{\overset{k_r}{\rightleftharpoons}} U^- + H_2O \qquad (2)$$

can be definitely ruled out on the following basis: (a) The relaxation amplitude should vary with the total monoanion concentration ($\bar{C}_{U^-}$) and also with the hydroxyl ion concentration ($\bar{C}_{OH^-}$), but in the latter case this is not observed. (b) The relaxation times should follow the equation

$$\tau^{-1} = k_r(\bar{C}_{OH^-} + \bar{C}_{UH_2}) + k_f \qquad (3)$$

where $k_f = k_r(\bar{K}_W/\bar{K}_1)$.[19] Identification of eq 3 with eq 1 would then lead to

$$k_r = k_{OH^-} = k_{UH_2} = k_0(\bar{K}_1/\bar{K}_W) \qquad (4)$$

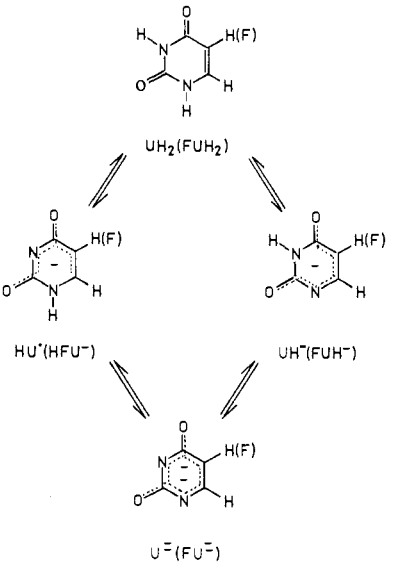
However, for both uracil and 5-fluorouracil, k_0, k_{UH_2}, and $k_0(\bar{K}_1/\bar{K}_W)$ differ among themselves by several orders of magnitude (Table II), and these differences are greater than the standard error in the data fitting procedure.

Thus, the observed relaxation does not arise from hydrolysis (eq 2). Nevertheless, some coupling with the tautomeric interconversion is expected, but is not detectable under our experimental conditions.

Kinetic Analysis of the Relaxation Time According to the "D Mechanism". Protonation of both UH^- and HU^- (or FUH^- and HFU^-) leads to the same neutral UH_2 (or FUH_2) molecule which deprotonates to give either tautomer (Scheme I). Likewise, deprotonation of both UH^- and HU^- leads to the same dianionic species U^{2-} (or FU^{2-}) which protonates to give either tautomer (Scheme I). The elementary protonation and deprotonation steps imply classical bimolecular proton exchange reactions (Scheme II) which are usually known[20] to be diffusion controlled when thermodynamically favored.

The reaction medium contains three acid species (the hydronium ion, water, and the neutral substrate UH_2 (or FUH_2)) and three basic species (the hydroxyl ion, water, and the substrate dianion U^{2-} (or $FU)^{2-}$. Therefore, the D mechanism implies six different reactions pathways[3] (Scheme II). Within the pH limits adopted here, and on the basis of knowledge of the concentrations ($\bar{C}_i$) and of the expected rate constants,[3,20] deprotonation of the monoanion by the dianion (pathway IIb) is negligible in comparison to that by the hydroxyl ion (pathway Ib); protonation by the proton (pathway Ia) is negligible compared to that by UH_2 (or FUH_2) (pathway IIa); similarly,

Scheme I



the base catalysis by water (pathway IIIb) is negligible compared to the acid catalysis by water (pathway IIIa). We therefore limit our discussion to pathways Ib, IIa, and IIIa).

The inverse of the relaxation time is expressed by

$$\tau^{-1} = \sum(k_n^+ + k_n^-) \qquad (5)$$

where k_n^+ and k_n^- are the direct and reverse first-order rate constants for the reaction along pathway "n". The steady-state approximation[21] applied to the dianion (U^{2-}) in pathway Ib and to the neutral molecule (UH_2) in pathway IIIa, followed by identification of eq 5 with eq 1, allows associating the $k_{OH^-}\overline{C}_{OH^-}$ term with pathway Ib, the $k_{UH_2}\overline{C}_{UH_2}$ term with pathway IIa, and the k_0 term with pathway IIIa.

Base Catalysis by the Hydroxyl Ion (Pathway Ib). The steady-state approximation applied to U^{2-} leads to

$$k_{OH^-} = \frac{b_1b_2 + b_{-1}b_{-2}}{b_2 + b_{-1}}$$

where b_1 and b_{-2} are the rate constants for deprotonation of the individual tautomers by the hydroxyl ion. The value of k_{OH^-} lies between b_1 and b_{-2}, and it is reasonable to assume that $b_1 \simeq b_{-2} \simeq k_{OH^-}$. The values of 3.9×10^8 and 3.85×10^9 M^{-1} s^{-1}, given respectively for U^- and FU^- (Table II), are to be compared with the rate constants for the deprotonation of anions such as hydrogen carbonate[20] or phosphate[21] by OH^-, while keeping in mind that rate constants become smaller for intermolecular proton transfer in which the free-energy change becomes smaller than 3 or 4 pK units.[3,22] It should be noted that k_{OH^-} is about ten times smaller for U^- than for FU^- whose pK_2 values are higher than 13.5 and equal to 12.6, respectively (Table I).

Acid Autocatalysis (Pathway IIa). The values of k_{UH_2} and k_{FUH_2}, given in Table II, compare favorably with rate constants found for similar symmetric proton exchanges, i.e., 3×10^8 and 1.0×10^8 M^{-1} s^{-1} for isocytosine[3] and the purine/purinate system,[23] respectively.

Acid Catalysis by Water (Pathway IIIa). The steady-state approximation applied to UH_2 (or FUH_2) leads to[3]

$$k_0 = a_{45}(\overline{K}'_{UH^-} + \overline{K}'_{HU^-})/2, \text{ where } \overline{K}'_{UH^-} = \frac{\overline{C}_{UH_2}\overline{C}_{OH^-}}{\overline{C}_{UH^-}};^{19}$$

$$\text{and} \quad a_{45} = 2\frac{a_{-4}a_5}{a_{-4} + a_5}$$

where a_{-4} and a_5 are the rate constants for deprotonation of the neutral UH_2 (or FUH_2) molecule by OH^-. The value of a_{45} lies between a_{-4} and a_5 and, once again, it is reasonable to assume that $a_{-4} \simeq a_5 \simeq a_{45}$ since the reaction of the hydroxyl ion with a "normal" acid is diffusion controlled.[20]

Using the values of k_0 given in Table II to estimate a_{45}, we obtain 1.16×10^{10} and 1.0×10^{10} for uracil and 5-fluorouracil, respectively. These values are to be compared with the previous estimates[6a,23] for the reaction of the neutral uracil molecule with OH^- which gives either monoanion (eq 2); we then have $k_r = a_{-4} + a_5 = 2a_{45}$ which makes

$$k_r = 2.3 \pm 0.3 \times 10^{10}\ M^{-1}\ s^{-1} \text{ for uracil}$$

$$k_r = 2.0 \pm 0.2 \times 10^{10}\ M^{-1}\ s^{-1} \text{ for 5-fluorouracil}$$

Chang and Grunwald[6a] estimate k_r to be 1.2×10^{10} for uracil. However, their estimation is based on the kinetic study of NH proton exchange between UH_2 and water, and on the assumption that the tautomeric interconversion of UH^- to

Scheme II. General Acid-Base Catalysis Model for Tautomeric Interconversion: "D Mechanism"

Pathway no.	Pathway	Catalyst	Contributions to the kinetic law
Ia	$UH^- + H^+ \underset{a_{-1}}{\overset{a_1}{\rightleftharpoons}} UH_2 \underset{a_{-2}}{\overset{a_2}{\rightleftharpoons}} HU^- + H^+$	H^+	$\frac{a_1a_2 + a_{-1}a_{-2}}{a_2 + a_{-1}}\overline{C}_{H^+}$
IIa	$UH^- + UH_2 \underset{a_{-3}}{\overset{a_3}{\rightleftharpoons}} HU^- + UH_2$	UH_2	$(a_3 + a_{-3})\overline{C}_{UH_2}$
IIIa	$UH^- + H_2O \underset{a_{-4}}{\overset{a_4}{\rightleftharpoons}} UH_2 + OH^- \underset{a_{-5}}{\overset{a_5}{\rightleftharpoons}} HU^- + H_2O$	H_2O	$\frac{a_4a_5 + a_{-4}a_{-5}}{a_5 + a_{-4}}$
Ib	$UH^- + OH^- \underset{b_{-1}}{\overset{b_1}{\rightleftharpoons}} H_2O + U^{2-} \underset{b_{-2}}{\overset{b_2}{\rightleftharpoons}} HU^- + OH^-$	OH^-	$\frac{b_1b_2 + b_{-1}b_{-2}}{b_2 + b_{-1}}\overline{C}_{OH^-}$
IIb	$UH^- + U^{2-} \underset{b_{-3}}{\overset{b_3}{\rightleftharpoons}} HU^- + U^{2-}$	U^{2-}	$(b_3 + b_{-3})\overline{C}_{U^{2-}}$
IIIb	$UH^- + H_2O \underset{b_{-4}}{\overset{b_4}{\rightleftharpoons}} U^{2-} + H_3O^+ \underset{b_{-5}}{\overset{b_5}{\rightleftharpoons}} HU^- + H_2O$	H_2O	$\frac{b_4b_5 + b_{-4}b_{-5}}{b_5 + b_{-4}}$

HU^- proceeds mainly through a ND mechanism (10^8 s^{-1}) which would imply that *both* NH protons of UH_2 exchange with water in each reversible cycle $UH_2 \rightleftharpoons UH^-$. If, as is done in this work, one rejects this assumption, the data obtained by these authors lead to a value of 2.4×10^{10} M^{-1} s^{-1} for k_r since only *one* exchangeable proton is exchanged in each cycle.

Yet Busse and Maass,[24] using the ultrasonic absorption method, estimate k_r to be 0.98×10^{10} M^{-1} s^{-1}. However, their technique seems to give rather imprecise results, as is illustrated by the discrepancies between their data on the deprotonation of amino acids[24] and that obtained by other authors.[25]

Indeed, deprotonation rates of neutral NH acids usually lie between 1.0 and 2.0×10^{10} M^{-1} s^{-1} when the acid only bears one deprotonatable site;[3,20,22] it is therefore reasonable to find a larger rate constant in the case of uracil, where there are two deprotonatable sites.

Conclusion

Laser temperature-jump spectroscopy has been used successfully in our study of the spectral changes following the heating of an aqueous uracil anion (U^-) solution. These changes occur in a single relaxation whose amplitude corresponds to the variations observed on a conventional spectrophotometer. This phenomenon, which we and others[14-18] attribute to tautomeric interconversion, occurs in the microsecond time range for uracil as expected with the "D mechanism". If the water exchange rates obtained by dynamic NMR were to be caused by a concerted proton-transfer tautomeric interconversion, then relaxations in the 0.01-μs time range should be observed. This is not the case (Figure 1). However, these observations might be explained as arising from a hydration equilibrium which others have shown to occur on another pyrimidine in this time range.[26]

Indeed, the "D mechanism" affords a very satisfactory picture for the tautomeric interconversion kinetics of uracil and 5-fluorouracil monoanions, as well as for cytosine and isocytosine. In these systems of very similar structure, observed relaxation times are separated by several orders of magnitude, whereas the kinetic constants are practically the same: the different pKs make the difference.

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References and Notes

(1) This article will be included in O. Bensaude's Doctoral Thesis, Université Paris VII.
(2) M. Dreyfus, O. Bensaude, G. Dodin, and J. E. Dubois, *J. Am. Chem. Soc.*, **98**, 6338 (1976).
(3) O. Bensaude, M. Dreyfus, G. Dodin, and J. E. Dubois, *J. Am. Chem. Soc.*, **99**, 4438 (1977).
(4) For an example and an exhaustive discussion of the "D mechanism" see P. Schuster, K. Tortschanoff, and H. Winkler, *Z. Naturforsch. C*, **31**, 219 (1976); P. Schuster, P. Wolschann, and K. Tortschanoff in "Chemical Relaxation in Molecular Biology, Biochemistry and Biophysics", I. Pecht and R. Rigler, Ed., Springer-Verlag, West Berlin, 1977.
(5) The following abbreviations are used in this text: UH_2 and FUH_2 are the neutral forms of uracil and 5-fluorouracil, while U^- and FU^- are the corresponding monoanions; HU^- and UH^- are the two possible monoanions of uracil, resulting from dissociation of the N-3 and N-1 protons, respectively, with similar connotations for FU^-; U^{2-} and FU^- are the dianions of UH_2 and FUH_2.
(6) (a) K. C. Chang and E. Grunwald, *J. Am. Chem. Soc.*, **98**, 3737 (1976); *ibid.*, (b) E. Grunwald and D. W. Fong, *ibid.*, **94**, 7371 (1972); (c) E. Grunwald, C. F. Jumper, and S. Meiboom, *ibid.*, **85**, 522 (1963); E. Grunwald and S. Meiboom, *ibid.*, **85**, 2047 (1963); Z. Luz and S. Meiboom, *ibid.*, **85**, 1425 (1963).
(7) J. Aubard, J. J. Meyer, and J. E. Dubois, *Chem. Instrum.*, **8**, 1 (1977).
(8) J. J. Meyer and J. Aubard, *Rev. Sci. Instrum.*, **48**, 695 (1977).
(9) M. Dreyfus, G. Dodin, O. Bensaude, and J. E. Dubois, *J. Am. Chem. Soc.*, **97**, 2369 (1975).
(10) Analytical TLC on cellulose plates (Merck) were performed in solvent A (1-butanol–acetic acid–water, 4:1:5) and in solvent B (2-propanol–aqueous ammonia–water, 7:1:2). All compounds were homogeneous in these conditions.
(11) Contribution of a $k_{H^+}\bar{C}_{H^+}$ term in eq 1a and 1b is negligible because the diffusion-controlled protonation of U^- implies that $k_{H^+} \sim 2 \times 10^{10}$ M^{-1} s^{-1}, thus $k_{H^+}\bar{C}_{H^+}$ is less than 2×10^3 s^{-1} above pH 7.
(12) J. J. Christensen, J. H. Rytting, and R. M. Izatt, *J. Chem. Soc. B*, 1643 (1970).
(13) D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952).
(14) K. Nakanishi, N. Suzuki, and P. Jamazaki, *Bull. Chem. Soc. Jpn.*, **34**, 53 (1961).
(15) K. L. Wierzchowski, E. Litonska, and D. Shugar, *J. Am. Chem. Soc.*, **87**, 4621 (1965).
(16) I. Wempen and J. J. Fox, *J. Am. Chem. Soc.*, **86**, 2474 (1964).
(17) A. Psoda, Z. Kazimierczuk, and D. Shugar, *J. Am. Chem. Soc.*, **96**, 6832 (1974).
(18) R. Shapiro and S. Kang, *Biochim. Biophys. Acta*, **232**, 1 (1971).
(19) $K_W = \bar{C}_{OH^-}\bar{C}_{H^+}$, $K_1 = \bar{C}_{U^-}\bar{C}_{H^+}/\bar{C}_{UH_2}$, $K'_{HU^-} = \bar{C}_{UH_2}\bar{C}_{OH^-}/\bar{C}_{HU^-}$. These equilibrium constants may be computed from the thermodynamic constants taking into account the activity coefficients from the Debye–Hückel equation: see R. G. Bates in "Determination of pH—Theory and Practice", 2nd ed, Wiley-Interscience, New York, N.Y., 1973, p 48. We used an ion-size parameter, $\mathring{a} = 9$ Å for H^+, and $\mathring{a} = 3$ Å for other ions at 0.2 M ionic strength. For neutral molecules, activity coefficients were set to unity.
(20) M. Eigen, *Angew. Chem., Int. Ed. Engl.*, **3**, 1 (1964).
(21) A more refined mechanism could be examined if one rejects the use of the steady-state approximation on UH_2 (see the appendix of ref 2). However, in this study the refined treatment did not significantly alter the values of the elementary kinetic constants. Therefore the steady-state approximation remained valid within the experimental errors.
(22) D. Grimshaw and E. Wyn-Jones, *J. Chem. Soc., Faraday Trans. 2*, **69**, 168 (1973).
(23) T. H. Marshall and E. Grunwald, *J. Am. Chem. Soc.*, **91**, 4541 (1969); for other examples, see also E. Grunwald and D. Eustace in "Proton-Transfer Reactions", E. Caldin and V. Gold, Ed., Chapman and Hall, London, 1975, Chapter 4.
(24) H. G. Busse and G. Maass, *Z. Phys. Chem. (Frankfurt am Main)*, **66**, 92 ((1969).
(25) D. Grimshaw, P. J. Heywood, and E. Wyn-Jones, *J. Chem. Soc., Faraday Trans. 2*, **69**, 756 (1973).
(26) D. Pörschke, *Rev. Sci. Instrum.*, **47**, 1364 (1976); see also D. Pörschke, *Biochemistry*, **15**, 1495 (1976).