On the Hydrolytic Behavior of Tinidazole, Metronidazole, and Ornidazole

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Received 1 August 2002; revised 3 October 2002; accepted 21 November 2002

ABSTRACT: Using two UV-spectrophotometric methods, the hydrolysis of tinidazole was studied at pH 1.00–8.45 at 80°C. The reaction followed apparent first-order kinetics throughout the studied range. No kinetic salt effect was detected, indicating that at least one of the reacting partners forming the transition state has a charge of 0. The reaction rate macro constants M_1–M_4 were calculated to be $3.35 \times 10^{-2}\ M^{-1}\ h^{-1}$, $1.45 \times 10^{-2}\ h^{-1}$, $3.76 \times 10^{-6}\ M\ h^{-1}$, and $2.85 \times 10^{-11}\ M^2\ h^{-1}$, respectively. At pH $\geq$ 7, the uncharged tinidazole was decomposed by the hydroxide ion; the reaction was found out to involve a proton transfer from the ethylsulfonylethyl side chain. At around pH 4.5, the degradation of the uncharged tinidazole was due to the solvent. In more acidic conditions, the reaction mechanism could not be fully resolved. The alkaline hydrolysis of metronidazole was studied on the basis of literature data. A general reaction mechanism was proposed, but an unequivocal explanation for the inflection point in the pH rate profile at pH 6 could not be found. The implications of the proposed reaction mechanism for the hydrolytic behavior of ornidazole were discussed. J Pharm Sci 92:739–746, 2003

Keywords: 5-nitroimidazoles; hydrolysis; degradation kinetics; degradation mechanisms; UV spectrometry

INTRODUCTION

Used against trichomoniasis, giardiasis, and amoebiasis, tinidazole (**1**) (1-[2-(ethylsulfonyl)ethyl]-2-methyl-5-nitroimidazole) is susceptible to both hydrolysis and photolysis. It is known to decompose hydrolytically in alkaline conditions to the 4-nitro isomer (**2**) and 2-methyl-4(5)-nitroimidazole (**3**) (Figure 1);[1] the molar amount of 5-nitroimidazole species is conserved in the reaction.[2] These decomposition products are also the only specifically named structurally related impurities limited by the monograph of **1** in the European Pharmacopoeia.[3] In acidic solutions, however, **1** is the only 5-nitroimidazole species present despite extensive degradation.[2] The pH rate profile of the pseudo first-order hydrolysis reaction of **1** at pH 1–12 has been determined but the degradation mechanisms could only be speculated on.[2]

Studies on the hydrolysis of two other 5-nitroimidazoles (Figure 1) have been reported as well. Wang and Yeh[4] studied the hydrolysis kinetics of metronidazole (**4**) (1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole) which has been previously[5,6] shown to loose the nitroimidazole structure in both acidic and alkaline media. A kinetic salt effect was detected at pH 3.1, while it was less pronounced at pH 7.4.[4] The defects in the mathematical treatment of the kinetic data have been pointed out and corrected by van der Houwen et al.[7] However, the corrected pH rate profile contains certain features that have apparently gone undetected by the previous authors.[7]

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Journal of Pharmaceutical Sciences, Vol. 92, 739–746 (2003)

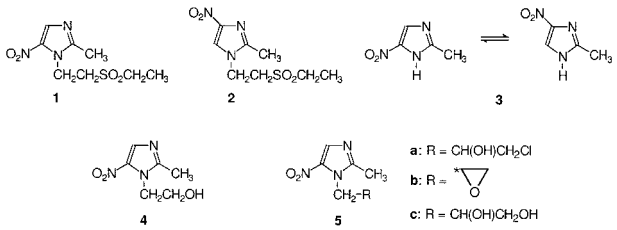


Figure 1. The chemical structures of tinidazole (**1**), its 4-nitro isomer (**2**), the tautomeric 2-methyl-4(5)-nitroimidazole (**3**), and metronidazole (**4**) as well as ornidazole (**5a**) and its epoxide (**5b**) and diol (**5c**) degradation products.

Both reactions being well-known organic chemistry, the alkaline hydrolysis (pH 6–10) of ornidazole (**5a**) (1-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole) has been shown to produce an epoxide (**5b**) from the side-chain chlorohydrin, and subsequently the corresponding diol (**5c**).[8,9] On the other hand, **5a** has also been reported[9] to loose the nitroimidazole structure in strongly acidic conditions (pH $\leq$ 1).

It is the aim of the present study to shed some light on the mechanisms of both the alkaline and acidic hydrolysis of **1**. Further, based on the present kinetic results for **1** and those previously reported for **4**,[4,5,7] we propose a more detailed mechanism for the alkaline hydrolysis of both drugs. These results will be shown to have some implications regarding the hydrolysis of **5a** also.

EXPERIMENTAL

Materials

Tinidazole (**1**) was generously supplied by Orion Pharmaceutica (Espoo, Finland). The commercially available 2-methyl-4(5)-nitroimidazole (**3**) was from Aldrich-Chemie (Steinheim, Germany). The identity and purity of these compounds had been verified by TLC, UV, IR, as well as by ^{1}H and ^{13}C-NMR spectrometry. The 4-nitro isomer of **1** (**2**) was prepared by the current authors as described in the literature;[10] its identity and purity were verified by HPTLC as well as by UV and IR spectrometry by comparing with a batch of known[11,12] identity. All other chemicals and reagents were of analytical grade. Sodium salts were used for the phosphate buffers; the ionic strengths were adjusted with sodium chloride.

Apparatus

The UV-spectrophotometric determinations were carried out using a Unicam UV2-300 spectrophotometer (Unicam Ltd., Cambridge, UK). pH measurements were performed at ambient temperature with a Radiometer PHM83 Autocal pHmeter (Radiometer A/S, Copenhagen, Denmark) equipped with a standard glass-calomel combination electrode or with a WTW inoLab pH Level 1 equipped with a WTW pH-Electrode SenTix 81 (WTW, Weilheim, Germany); commercial pH standard buffers (pH 1.09, 1.679, 4.005, 7.000, and 10.00) were used as appropriate.

Kinetic Results at Varying Ionic Strengths

Because the ionization constants of phosphoric acid are practically temperature-insensitive,[13] the pH of the solutions was adjusted at room temperature exactly to the value shown by potentiometrically titrating one 0.1 M buffer component with the other. The pH of the hydrolyzed solutions was not changed by more than $\pm$0.02 pH units during the test period, which could be as long as 2 months. Several solutions of varying ionic strengths ($n = 4$–5) were studied at each pH. Only the degradation kinetics of tinidazole was followed (cf. Results and Discussion). The order of the reaction was decided on the basis of the correlation coefficient, the residual standard deviation and the magnitude and visually estimated distribution of the residuals.

pH 1.00–4.70

The ionic strengths varied from approximately 0.1 to 1 (however, to 0.4 at pH 4.00). Solutions of **1** (0.6 mM) were prepared in 0.1 M phosphate buffer of appropriate pH by dissolving the solid in 100 mL of the buffer solution at room temperature in 200-mL volumetric flasks, which were then placed in an oven kept at 80 $\pm$ 0.5°C. Seven aliquots in total, 5.0 mL aliquots of the hydrolyzed solution were drawn at intervals and added to 10 mL of 0.1 M phosphate buffer in an ice-water bath. After cooling to ambient temperature, the mixture was diluted to 25.0 mL with the same buffer (pH appropriate to ensure a final pH of 6.0 $\pm$ 0.7[14]). The absorbances of the samples were measured at 318 nm against a reagent blank using a spectral bandwidth of 1.5 nm and an integration time of 1 s. Made by diluting 240–600 μL (60-μL increments) of a 5 mM stock solution of **1** in water to 25.0 mL with a 0.1 M

phosphate buffer (pH 6), seven freshly prepared standards were analyzed with the samples. A second-order polynomial, $A = a_0 + a_1c + a_2c^2$, where A is the absorbance of the solution and c the concentration of **1**, was used for the calibration.[14]

pH 7.00–8.45

The ionic strengths varied from approximately 0.2 to 1 (however, to 0.6 at pH 8.45). Solutions of **1** (0.6 mM) were prepared in 0.1 M phosphate buffer of appropriate pH (7.50–8.45) by dissolving the solid in 95 mL of the buffer solution at $80 \pm 0.5^{\circ}$C in a 100-mL volumetric flask submerged in a water bath kept at the same temperature. Seven aliquots in total, 10.0 mL aliquots of the hydrolyzed solution were drawn at intervals and added to 5 mL of 0.1 M phosphate buffer in an ice-water bath. After cooling to ambient temperature, the mixture was diluted to 20.0 mL with the same buffer (pH appropriate to ensure a final pH of 6.0 ± 0.7[14]). Additionally, 0.6 mM tinidazole solutions at pH 7.00 were prepared in 0.1 M phosphate buffer by dissolving the solid in 100 mL of the buffer solution at room temperature in 200-mL volumetric flasks, which were then placed in an oven kept at $80 \pm 0.5^{\circ}$C. Diluted with 0.1 M phosphate buffer (pH 5.00), the aliquots were drawn and handled as described above.

The spectra of the samples were recorded between 240–290 nm at 2.0 nm intervals, i.e., at 26 wavelengths, against a reagent blank using a spectral bandwidth of 1.5 nm and a scan speed of 30 nm/min. For the quantitations, the absorbances at six wavelengths, namely 240, 242, 246, 248, 254, and 270 nm, were used.[14] Made from 10 mM stock solutions in water by diluting with 0.1 M phosphate buffer (pH 6), nine freshly prepared standards (Table 1) were analyzed with the samples. The results were calculated by finding the least squares solution[15] to the matrix equation $A = KC$, first for K and then for C, where A, K, and C are the matrices of absorbances, proportionality constants (ab of the Lambert-Beer equation), and concentrations, respectively.

Molecular Modeling

The calculations were performed with Spartan 5.0.3 (Wavefunction Inc., Irvine, CA) at the pBP/DN**//HF/3-21G$^{(*)}$ level of calculation, run on an SGI R5000 processor (SGI, Mountain View, CA).

RESULTS AND DISCUSSION

The hydrolysis kinetics of **1** followed apparent first-order kinetics in all the studied cases. At pH 7.00–8.45, the molar concentration of total 5-nitroimidazoles present in the solution was practically constant. The amount of **3** grew steadily in the hydrolyzed solutions, yet not according to either zero- nor first-order kinetics. Lastly, the concentration of **2** was always below the quantitation limit[14] and many times even below the detection limit[14] in all sample solutions studied.

Tinidazole (1)

Kinetic Salt Effect

Although the hydrolysis rate seemed to vary with changing ionic strength (μ), only at pH 1.50 and 2.50 was there a statistically significant ($\alpha = 0.05$) dependence on

$$\mu_{DH} = \frac{\sqrt{\mu}}{1 + 1.6 \times \sqrt{\mu}}$$

of the logarithm of the rate constant. (Although the modified Debye-Hückel equation is usually taken to hold up to about $\mu = 0.1$, Carstensen[16] has discussed and exemplified the applicability of μ_{DH} for ionic strengths as high as 1, in interpreting the kinetic salt effect.) The slopes were 0.0854 (pH 1.50) and 0.0457 (pH 2.50), whereas the theory[16] would predict a value of $2Qz_Az_B = 1.145z_Az_B$ at 80°C, z_A and z_B being the charges of the reacting species. It is, therefore, safe to state that even in these cases at least one of z_A and z_B is 0 in the major degradation pathway. Thus, it may be deduced that at least one of the

Table 1. The Concentrations (mM) of **1–3** in the Standard Solutions (S1–S9)

Compound	S1	S2	S3	S4	S5	S6	S7	S8	S9
1	0.329	0	0	0.0301	0.0602	0.209	0.0451	0.151	0.106
2	0	0.330	0	0.210	0.0302	0.0599	0.106	0.0448	0.151
3	0	0	0.330	0.0599	0.211	0.0301	0.151	0.105	0.0450
Σ	0.329	0.330	0.330	0.300	0.301	0.299	0.302	0.300	0.302

reacting partners contributing to the transition state in the hydrolysis of **1** at pH 1.00–8.45 has a charge of 0.

Kinetic Parameters

1 has one ionizable group (N^3 of the imidazole ring) with a pK_a value of 1.82,[17] and therefore,[18] its first-order hydrolysis rate constant is described by

$$k_{obs} = \frac{M_1 \times [H^+]^2 + M_2 \times [H^+] + M_3 + M_4 \times [H^+]^{-1}}{[H^+] + K_a} \quad (1)$$

where M_1–M_4 are the so-called macro constants, each combining kinetically indistinguishable reaction rate constants:

$$M_1 = k_{0,H} \quad (2)$$

$$M_2 = k_{1,H} \times K_a + k'_{0,S} \times [H_2O] = k_{1,H} \times K_a + k_{0,S} \quad (3)$$

$$M_3 = k'_{1,S} \times K_a \times [H_2O] + k_{0,OH} \times K_w = k_{1,S} \times K_a + k_{0,OH} \times K_w \quad (4)$$

$$M_4 = k_{1,OH} \times K_a \times K_w \quad (5)$$

The individual reaction rate constants take the form $k_{n,N}$, where $n = 0,1$ states the number of dissociated protons in [tinidazole + H]$^+$, whose K_a appears in the equations, and N = H,S,OH denotes the other reacting species, H^+, H_2O (or solvent), and OH^-, respectively. Because **1** is an uncharged base, $k_{0,H}$ and $k_{0,OH}$ describe reactions with both reacting partners charged (exhibiting kinetic salt effect), whereas $k_{1,H}$, $k_{0,S}$, $k_{1,S}$, and $k_{1,OH}$ describe reactions with at least one uncharged partner (fairly immune to varying ionic strength).

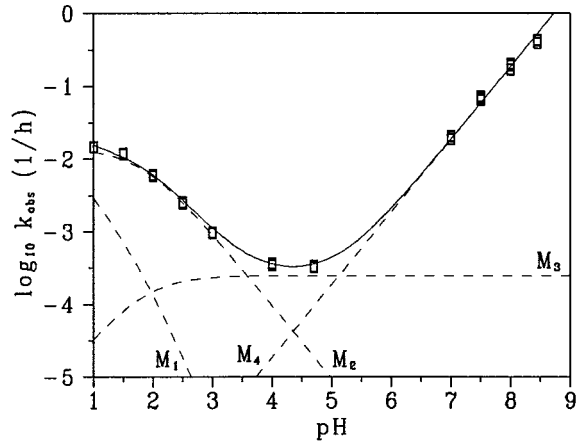


Figure 2. The reaction rate profile of tinidazole (**1**) with the individual contributions of the macro constants.

Using the parameter estimation described by Some et al.[19–21] (simplex optimization was replaced by Dataplot's[22] command FIT), the "best-fit" estimates for M_1–M_4 were calculated by regarding the tests at varying ionic strengths as mere replicate determinations at each pH (Figure 2). Based on the values in Table 2, M_1 would contribute to k_{obs} by approximately 20% at pH 1.00 and by 7% at pH 1.50. One would, therefore, have also expected to see the kinetic salt effect at pH 1.00, with a slope of 0.2 (providing M_2 experiences only a slight salt effect if any). On the other hand, the contribution of M_1 to k_{obs} is so small that the

Table 2. Macro Rate Constants (± Associated Standard Deviations) According to Equations 1[18] and 6[18,29] for the Hydrolysis of **1** at pH 1.00–8.45 (80°C) and **4** at pH 3.1–9.9 (90°C)[4]

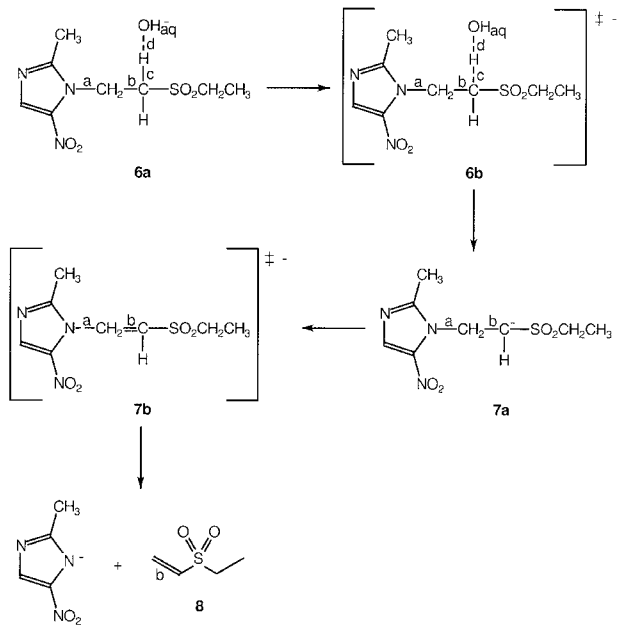
Macro Constant	Tinidazole (**1**) (eq. 1)	Metronidazole (**4**)	
		Eq. 1	Eq. 6[c]
M_1 (M^{-1} h^{-1})	$3.35 \times 10^{-2} \pm 3.46 \times 10^{-3}$	4.1	4
M_2 (h^{-1})	$1.45 \times 10^{-2} \pm 1.64 \times 10^{-4}$	4.3×10^{-4}	1×10^{-2}
M_3 (M h^{-1})	$3.76 \times 10^{-6} \pm 9.42 \times 10^{-8}$	2.9×10^{-9}	1×10^{-6}
M_4 (M^2 h^{-1})	$2.85 \times 10^{-11} \pm 2.33 \times 10^{-13}$	7.2×10^{-18}	2×10^{-14}
M_0^b (M^{-1} h^{-1})	—	—	1×10^{-3}
M_1^b (h^{-1})	—	—	2×10^{-8}
pK_a	1.82[17,a]	6.0[b]	2.55[25]

[a] Determined at 25°C.
[b] Approximated from kinetic data.
[c] pK_a = 6.0 used for the buffer species.

precision of the calculated value is poor, and it is difficult to demonstrate whether there is a salt effect or not. Based on these considerations, no decision could be made on the existence of $[\text{tinidazole} + \text{H}]^+ + \text{H}^+$. M_2 is composed of two rate constants (eq. 3), both of which are (almost) independent of the ionic strength; therefore, it is not possible to assign which reaction, or both, is responsible for the macro constant. On the other hand, M_3 has one component of both kinds. As can be seen from Figure 2, M_3 was the major component of k_{obs} at pH 4–5, and one would have expected to see a kinetic salt effect if $k_{0,OH} \times K_w$ (salt effect) was large enough as compared with $k_{1,S} \times K_a$ (no salt effect). Because no salt effect was detected, it was concluded that $k_{1,S} \times K_a$ dominates M_3 yielding $k_{1,S} = 2.48 \times 10^{-4}$ h^{-1} ($k'_{1,S} = 4.61 \times 10^{-6}$ M^{-1} h^{-1}; $[\text{H}_2\text{O}] = 53.9$ M at 80°C); from M_4, $k_{1,OH} = 8190$ M^{-1} h^{-1} ($K_w = 2.30 \times 10^{-13}$ M^2 at 80°C;[23] $\rho_w = 0.97183$ g cm^{-3}). Thus, it may be concluded that in alkaline conditions (pH ≥ 7) **1** is mainly decomposed as a result of the uncharged **1** reacting with the hydroxide ion, thereby producing **2** and **3**. In mildly acidic conditions (around pH 4.5), the course of the reaction is governed by solvent-assisted degradation of the nitroimidazole moiety in the uncharged **1**. In more acidic conditions, the nitroimidazole structure is decomposed as well. However, it could not be elucidated, whether it is the charged or uncharged **1** that reacts, or both.

Mechanism of Alkaline Hydrolysis

Based on the kinetic data and molecular modeling, the mechanism in Scheme 1 is proposed for the alkaline hydrolysis of **1** (Table 3). In a microhydrated environment in the gas phase, there existed a stable encounter pair between the hydrated hydroxide ion (4 H_2O)[24] and **1** (**6a**). Of the four available hydrogens on the C^1 and C^2 atoms of the side chain, only the ones on the imidazole "methyl side" of the side chain were capable of taking part in an encounter pair with the hydrated hydroxide ion. Of these, only the encounter pair with the "methyl-side" hydrogen on C^2 was able to yield a transition state (**6b**) describing the proton transfer to the hydroxide ion. The deprotonated **1** (**7a**) was then able to decompose to ethyl vinyl sulfone (**8**) and the deprotonated **3** through another transition state (**7b**). It was not possible to reproduce the energetics of the hydrolysis reaction at the used calculation level. Also, the imaginary frequency of both transition



Scheme 1.

states described exaggerated atomic movements, yet the calculations verified the possible existence of the proposed reaction scheme. Although Scheme 1 involves a cascade of reactions, it cannot be excluded that the proton transfer and the formation of **8** may take place simultaneously. As a final remark, it must be noted that the $\text{p}K_a$ value associated with the proton transfer is so high (around 14–15), i.e., well outside the studied pH range, that it may be excluded from the kinetic equations.[7]

Metronidazole (4)

An approximate fit of eq. 1 to the original data[4] is shown in Figure 3a, which is essentially

Table 3. Calculated (HF/3-21G(*)) Bond Lengths Relevant for Scheme 1

	Bond Length (Å)			
Compound	a	b	c	d
1	1.47	1.54	1.08	—
6a	1.48	1.54	1.08	1.94
6b	1.49	1.54	1.57	1.10
H_2O	—	—	—	0.97
7a	1.58	1.46	—	—
7b	1.78	1.41	—	—
8	—	1.31	—	—

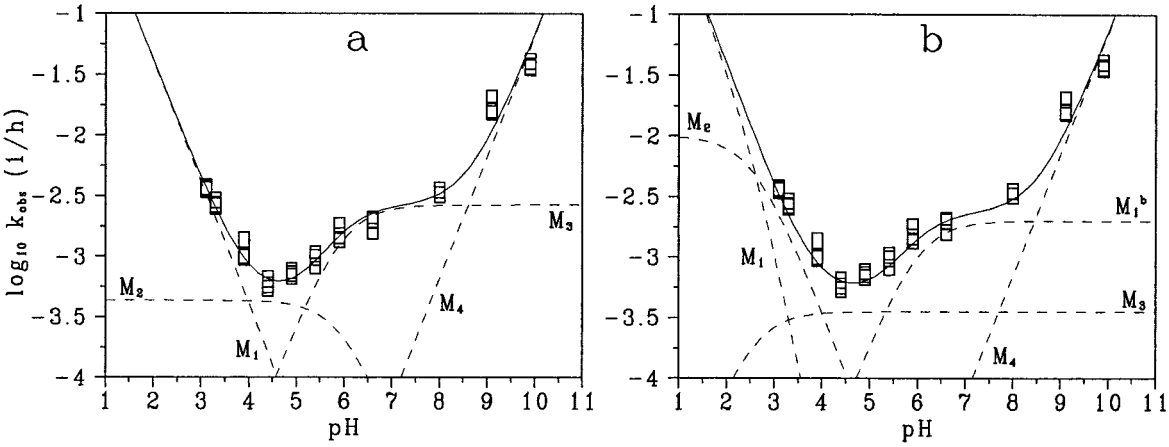
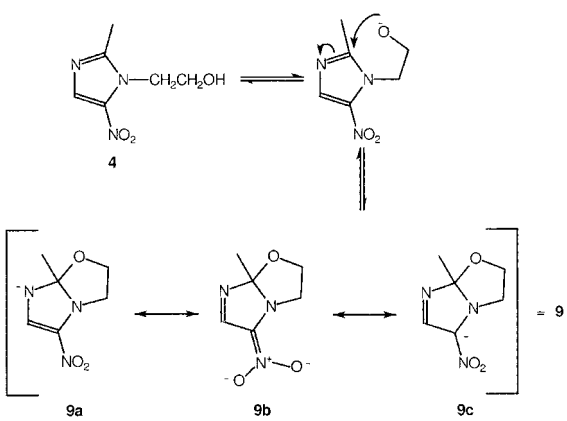


Figure 3. The reaction rate profile of metronidazole (**4**) based on previous studies[4,7] according to eqs. 1 (**a**) and 6 (**b**).

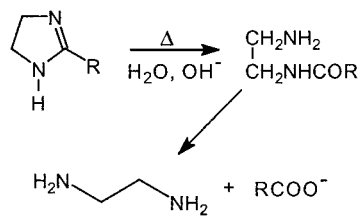
equivalent to the previous[7] graph; the data did not lend itself to an iterative fit, and the reported parameter values (Table 2) are the result of a visual fitting. (It must be noted that the original data[4] contains no data points below pH 3.1, and one must be very skeptical towards the pH rate profile (Figure 3a) at low pH.) It is apparent from eq. 1 that the contribution of M_3 reveals the approximate pK_a value of the degrading species quite easily; Figure 3a was produced using a pK_a value of 6.0, which is in sharp contrast with the literature[25] value of 2.55 for **4**. Although several explanations may be possible, two putative mechanisms seem to provide useful information.

A speculative reaction mechanism for the alkaline hydrolysis of **4** is depicted in Scheme 2, the



Scheme 2.

degrading species being an imidazoline (**9**), which would be susceptible to partial hydrolysis by hot water and to complete hydrolysis in alkali (Scheme 3).[26] **4** being in dynamic equilibrium with **9** at elevated temperatures, one would still be able



Scheme 3.

to monitor the reaction by analyzing the amount of unreacted **4**. Scheme 2 involves the deprotonation of the side-chain hydroxyl (p$K_a \approx 14.4$[27]) followed by ring closure, thereby leading to the reversible destruction of the aromatic imidazole structure, an obvious reason for the markedly slower reaction rate in alkaline conditions with respect to **1**;[2] one would expect the equilibrium to lie heavily in favor of the stable aromatic imidazole structure. Additionally, this 5-*Endo-Trig* ring closure is a disfavored reaction according to the Baldwin rules.[28] At the used calculation level, it was not possible to produce the transition state leading to **9**, which was a stable structure in the gas phase. According to the modeling results, the structure of **9** closely resembles that of **9b**; the calculated bond lengths and bond orders suggest the degradation of the imidazole structure and the emergence of the distinctly shorter (double) bonds at the sites indicated. However, Scheme 2 can represent the general mechanism of the alkaline hydrolysis at the most. Because a dynamic equilibrium is involved and the expected amount of **9** would be very small, the proposed mechanism cannot

explain the difference in pK_a values, which was pointed out. Apart from the degrading species itself, another potential factor causing inflection points in the pH rate profile at a pH corresponding to a pK_a value is the buffering agent.[29] The present data[4] at pH 5.4–8.0 was acquired in 0.1 M phosphate whose $pK_{a,2} = 7.09$;[30] eq. 6 is the representation of k_{obs} for this limited range including the macro constants M_0^b and M_1^b for buffer catalysis:[18,29]

$$k_{obs} = \frac{M_1 \times [H^+]^2 + M_2 \times [H^+] + M_3 + M_4 \times [H^+]^{-1}}{[H^+] + K_a} + \frac{M_0^b \times [H^+] + M_1^b}{[H^+] + K_{a,b}} \times [\text{buffer}] \quad (6)$$

It is seen from Figure 3b (Table 2) that eq. 6 may be fitted to the data[4] visually using a $pK_{a,b}$ of 6.0; M_0^b is small and does not show in the figure. Buffer catalysis as an explanation for the inflection point, however, contradicts the original results and conclusions.[4] Therefore, further studies are needed to elucidate the origin of the inflection point and the reaction mechanisms of **4**.

The proposed hydrolysis mechanism for **4** (Scheme 2) would imply that **5a**, after having degraded to **5c**, would also take part in a similar reaction. There would exist possibilities for both a disfavored 5- and a favored 6-*Endo-Trig* ring closure.[28] However, according to the modeling results, the cyclization itself would be endothermic in both cases, while it is slightly exothermic for **4** in the gas phase. Therefore, the applicability of Scheme 2 for reactions of **5c** cannot be confirmed. In any case, the disintegration of the aromatic ring structure is not capable of competing with the formation of **5b** and **5c**, because the latter reaction is four to five orders of magnitude faster at 60°C than the decomposition of **4** at 90°C.[4,8] Also, it is highly possible that **1, 4** and **5a** may have a similar, although not common, decomposition pathway in acidic conditions involving the degradation of the nitroimidazole moiety.

ACKNOWLEDGMENTS

We wish to thank Ms. Inkeri Huttunen for carrying out the synthesis of **2** and the reviewers for helpful comments. J.-P.K.S. wishes to acknowledge the financial support by the Academy of Finland through the Graduate School in Pharmaceutical Research (Ministry of Education, Finland).

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