Isoxazoles. 10. Degradation and Enolization Kinetics of 4-Aminoisoxazolyl-1,2-naphthoquinone in Basic Aqueous Solution

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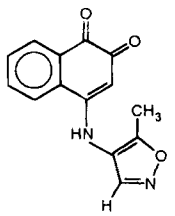
Abstract □ The kinetics of enolization and degradation of *N*-(5-methyl-4-isoxazolyl)-4-amino-1,2-naphthoquinone (**1**) was investigated in aqueous solutions over a pH range of 7.30 to 12.25, at 35 °C and at constant ionic strength ($\mu = 0.5$) using reversed-phase HPLC. Pseudo-first-order kinetics was observed throughout the pH range studied. The rate of enolization (k_e), the keto–enol equilibrium constant (K_t), and specific base catalysis rate constant (k_{OH}) were determined. Good agreement between the theoretical pH–rate profile and the experimental data supports the proposed transformation process. The average recovery for **1** and its tautomerization product 2-hydroxy-*N*-(5-methyl-4-isoxazolyl)-1,4-naphthoquinone 4-imine (**2**) from mixtures of different composition was evaluated.

Introduction

There are few studies in the literature about the keto–enol tautomerism in naphthoquinones derivatives,[1,2] so we considered it of interest to study the behavior of *N*-(5-methyl-4-isoxazolyl)-4-amino-1,2-naphthoquinone (**1**) in a wide pH



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range. This isoxazolylnaphthoquinone with an amine group in the 4-position of the isoxazole ring belongs to a relatively unexplored class of naphthoquinones[3] with important potential biological properties. In a previous report[4] on the chemical stability of **1** in acidic solutions, we determined the specific rate constants k_H, k_o and pk_{a1}, at 35 °C. In this paper we extended the kinetic studies in order to get information about the reactivity of **1** in basic aqueous solution for a better understanding of keto–enol interconversion in isoxazolylnaphthoquinones.

Experimental Section

Materials—All chemicals and reagents were analytical grade. Water used for buffers and the mobile phase in HPLC was purified according to a technique previously reported,[4] and methanol was treated with 2,4-dinitrophenylhydrazine according to the literature procedure.[5]

***N*-(5-methyl-4-isoxazolyl)-4-amino-1,2-naphthoquinone (1)** and **2-hydroxy-*N*-(5-methyl-4-isoxazolyl)-1,4-naphthoquinone 4-imine (2)** were prepared from sodium 1,2-naphthoquinone-4-sulfonate according to the method previously reported.[6]

2-Hydroxy-1,4-naphthoquinone (3) was purchased from Sigma Chemical Co. and was used as received.

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Table 1—Buffer Systems, Observed Rate Constants, t_{90}, and $t_{1/2}$ for the Degradation of 1 at 35 °C

pH	Buffer System	$10k_{obs}$, h^{-1} (CV)	t_{90}, h	$t_{1/2}$, h
7.32	$NaOH:KH_2PO_4$	0.0139 (5.2)	75.5	499
8.30	$NaOH:KH_2PO_4$	0.0357 (8.4)	29.4	194
8.95	$NaOH:NaHCO_3$	0.316 (6.3)	3.32	21.9
9.64	$NaOH:NaHCO_3$	1.97 (5.1)	0.534	3.52
10.00	$NaOH:NaHCO_3$	1.57 (1.3)	0.669	4.41
10.25	$NaOH:NaHCO_3$	1.84 (5.9)	0.571	3.77
10.90	$NaOH:NaHCO_3$	2.32 (5.4)	0.453	2.99
11.15	NaOH:KCl	5.01 (5.4)	0.210	1.38
12.25	NaOH:KCl	20.2 (1.2)	0.0522	0.35

***N*[1]-2-Pyrimidylsulfanilamide (4)** was analytical grade.

4-Amino-5-methylisoxazole (5) was prepared using methods described in the literature.[7,8]

Buffer Solutions—Buffer solutions[9] consisted of suitable mixtures of analytical grade potassium dihydrogen phosphate, sodium hydroxide, and sodium hydrogen carbonate (Table 1). The relative amount of the ionic species in the buffers was calculated from the equations given by Laitinen,[10] and a constant ionic strength (μ) of 0.5 was maintained by adding an appropriate amount of sodium chloride.

Internal Standard—A solution of *N*[1]-2-pyrimidylsulfanilamide (**4**) was used as an internal standard. It was accurately weighed (2.5000 mg), transferred to a volumetric flask (250 mL), and made up to volume with methanol. The solution was stored in the dark at 5 °C.

Apparatus—The modular chromatographic system consisted of a Konik 500 G pump, Rheodyne model 7125 20 μL loop injector, a UVIS-200 variable-wavelength UV detector operated at 264 nm and 0.032 AUFS, and a Konik model SP-4290 Integrator. A Hibar-Merck Lichrosorb C_{18} 10 μm column (250 × 4 mm) was used at a flow rate of 1.4 mL/min and at room temperature. The mobile phase, methanol: water (50:50 v/v), was filtered (0.45 μm Mylon-66 membrane) and deaerated prior to use. A pH meter (Orion Model SA 520) fitted with combination electrodes was used for all pH measurements. A Cahn Electrobalance model G with a sensitivity of 2×10^{-4} mg was used to weigh the samples. For kinetic measurements, the constant temperature bath was regulated by a Haake F_3 thermostat with ±0.1 °C precision.

Kinetic Procedure—The tautomerization and degradation rates were followed with the reversed phase HPLC procedure previously reported.[4] Accurately weighed **1** was transferred to a volumetric flask (25 mL) with the appropriate buffer, sonicated until completely dissolved, and then transferred to a tightly closed snap flask. The temperature was equilibrated at 35 °C and then the entire flask was submerged. Aliquots of the solutions with an 8×10^{-5} M concentration were withdrawn periodically, diluted to a known volume with internal standard solution, and then analyzed. The concentration of remaining **1** at time t was calculated from a peak-area ratio (drug/internal standard) and their conversion factor was obtained from the slopes of the corresponding calibration curve ($r = 0.998$, CV = 2.6). Triplicate samples were run for each storage condition.

Results and Discussion

Method Validation—Previously we have reported[6] the development of a simple, precise and specific HPLC procedure for the determination of **1** and its enol isomer 2-hydroxy-*N*-(5-methyl-4-isoxazolyl)-1,4-naphthoquinone 4-imine (**2**).

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Table 2—Average Recovery of Compounds 1 and 2

Amount Added, μg/mL		Nominal Value, %		Recovery, % (CV)	
1	2	1	2	1	2
12	28	30	70	31.3 (2.3)	62.5 (5.0)
20	20	50	50	49.40 (0.34)	45.10 (0.69)
28	12	70	30	71.2 (1.0)	28.7 (1.9)

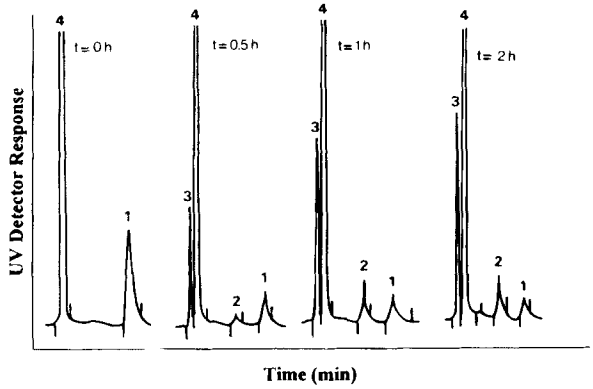


Figure 1—HPLC chromatograms of **1** at pH 12.25. The retention times of **1**, its degradation products **2** and **3**, and the internal standard **4** were 7.03, 4.73, 1.44, and 1.87 min, respectively.

In this paper, the average recovery for **1** and **2** in 30:70, 50:50, and 70:30 mixtures was determined respectively. Analyses in methanolic solutions were performed in triplicate and by direct comparison with standard samples of sulfadiazine (**4**). The results (Table 2) show a relative standard deviation of less than 2.3%, which indicates the effectiveness of the analytical method.

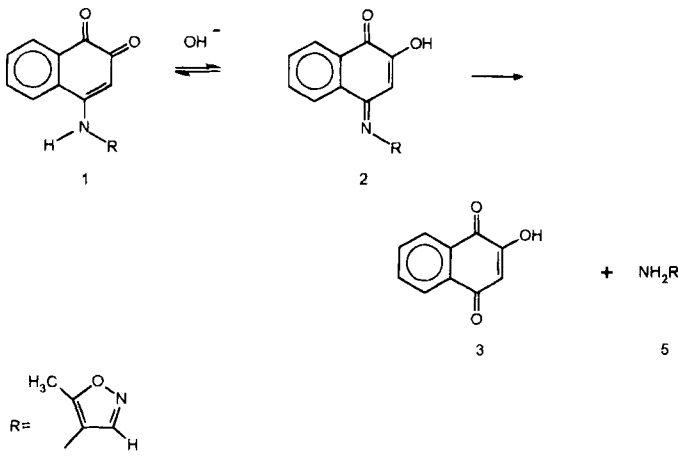
Identification of Reaction Products—The stability-indicating nature of the analytical method was evaluated using solutions of **1** which were stored at 25, 50, and 70 °C. Complex chromatograms were obtained at 50 and 70 °C, which indicates that the decomposition of **1** was notably accelerated with increase of temperature. Based on these results and those obtained at 25 °C, we undertook the study of the behavior of **1** at 35 °C in a wide pH range.

A typical HPLC scan of the reaction mixture in pH 12.25 (NaOH:KCl) at 35 °C is shown in Figure 1. The peak designated as **1** is the parent compound and those designated as **2** and **3** are its tautomer and the hydrolysis degradation product, 2-hydroxy-1,4-naphthoquinone, respectively. The reference standard was designated as **4**. Identification of products **2** and **3** is based on a comparison of the HPLC retention time with authentic samples.

The results obtained in the pH range studied show that compound **1** can react according to Scheme 1, to form 2-hydroxy-*N*-(5-methyl-4-isoxazolyl)-1,4-naphthoquinone 4-imine (**2**), 2-hydroxy-1,4-naphthoquinone (**3**), and 4-amino-5-methylisoxazole (**5**), depending on the solution pH.

Reaction Order and Observed Rate Constants—The disappearance of **1** followed apparent first-order kinetics at pH 7.30–12.25. These results are shown in plots of the logarithm of residual concentration of **1** versus time at 35 °C (Figure 2). The plots exhibited good linearity ($r > 0.99$) for experiments at all pH levels studied. The rate constants were computed by least-squares linear regression. The observed rate constants (k_{obs}), $t_{1/2}$, and t_{90} are given in Table 1.

Enolization Kinetics—Rates of enolization of **1** were measured by HPLC at 264 nm in buffer solutions (pH 7.32–10.25) at 35 °C, and the ionic strength was maintained at 0.5 constantly through the addition of NaCl. Under these conditions the reaction follows a pseudo-first-order process. Ob-



Scheme 1

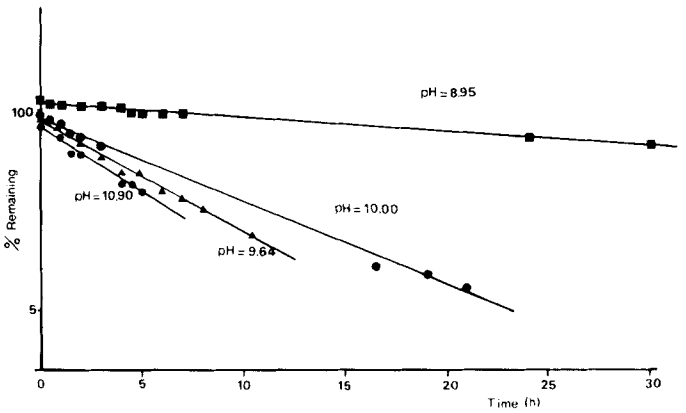


Figure 2—Pseudo-first-order degradation kinetics of **1** at various pH values, 35 °C, and $\mu = 0.5$.

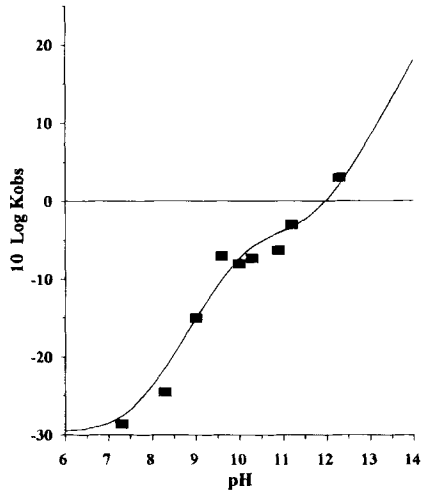
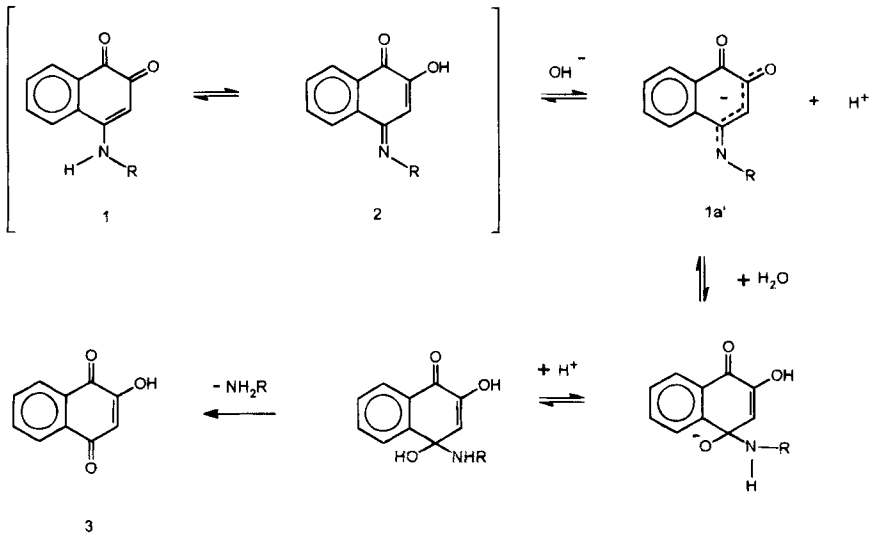


Figure 3—pH–rate profile of the degradation of **1** at 35 °C. The points are experimental values, and the solid line is the theoretical curve calculated from eq 1.

served rate constants were evaluated by linear least-squares analysis. The results summarized in Table 1 and displayed in Figure 3 show that the rate of enolization at pH 9.64–10.25 remains essentially constant and pH independent.

Keto–Enol Equilibria—The position of equilibrium was measured by monitoring the decrease in the concentration of **1** by HPLC.

pH–Rate Profile—The pH–rate profile at constant $\mu = 0.5$ and at 35 °C (Figure 3) shows that the apparent first-



Scheme 2

order rate constant for enolization of **1** increases linearly with increasing pH from 7.32 to 9.64 and then slowly decreases from pH 9.64 to 10.25. The inflection at this pH range indicates that ionization of the enol has an effect on the rate which becomes less sensitive to pH changes.

Above pH 10.90 the observed rate of degradation (k_{OH}) increases uniformly with increasing pH. Moreover, HPLC monitoring of the reaction mixture under these conditions revealed that the reaction proceeded to yield **3** exclusively. Since slopes of the straight line portions of the profile are close to unity, these two regions are associated with specific base catalysis. A rate equation for the transformation of **1** as a function of pH can be written as follows:

$$k_{obs} = k_o + K_t\frac{Ka}{Ka + [H^+]} + K_t\frac{Ka}{Ka + [H^+]}k_{OH}[OH^-] \quad (1)$$

where k_{obs} is the overall observed rate constant, k_o is the spontaneous or water catalysis rate constant, K_t is the tautomerization constant, k_{OH} is the specific basic catalysis rate constant, and Ka/(Ka + [H$^+$]) is the fraction of the compound in the enolate anion form.

Rate constants were estimated from the best fit and from the experimental pH–rate profile obtaining the following parameters: $k_o = 1.34 \times 10^{-3}$, $K_t = 0.40$, $k_{OH} = 160\ M^{-1}\ h^{-1}$, and Ka = 8×10^{-11}. The good agreement between the calculated values and the experimental data demonstrates that this equation adequately describes the degradation kinetics in the pH range studied.

Possible Degradation Mechanism—The transformation of **1** was found to be pH dependent. At pH 7.32–10.25, the predominant reaction was keto–enol tautomerism.

In the region between pH 10.90 and 12.25, the hydrolysis process occurs through a specific basic catalysis. On the basis of the products obtained and the data found in the literature on the reactivity of naphthoquinone derivatives,[11–14] we can assume an enol–enolate equilibrium given by the proton transfer reaction shown in Scheme 2. The intermediate **1a'** yields the isolated products **3** and **5** by addition of water, recombination with the proton, and subsequent cleavage of the C–N.

Conclusion

Compound **1** is a new methylated isoxazolylnaphthoquinone with the amine group in the 4-position of the isoxazole ring. It undergoes transformation in aqueous alkaline solutions which involves a keto–enol tautomerism to give its enol isomer 2-hydroxy-*N*-(5-methyl-4-isoxazolyl)-1,4-naphthoquinone 4-imine (**2**). This product experiences an enol–enolate equilibrium with subsequent hydrolysis on carbon 4 of the naphthoquinone ring to give 2-hydroxy-1,4-naphthoquinone (**3**), as observed by HPLC.

Since no other intermediate product was identified in the reaction course by HPLC, the obtained kinetic data, and the proposed mechanism indicate that the results and the expression rate are reasonable.

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