Isoxazoles. VII: Hydrolysis of 4-Methyl-5-isoxazolylnaphthoquinone Derivatives in Aqueous Solutions

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Abstract □ The kinetics for the degradation of 2-(4-methyl-5-isoxazolylamine)-*N*-(4-methyl-5-isoxazolyl)-1,4-naphthoquinone-4-imine (**1**) in solution were investigated at 70 °C and at a constant ionic strength of 0.5 over a pH range of 1.75 to 12.85. The degradation rates were determined by absorption and second-derivative UV spectrometry. Two degradation products were identified in acidic and neutral pHs; they are 4-*N*-(4-methyl-5-isoxazolyl)-1,2-naphthoquinone (**2**) and 2-methylcyanoacetamide (**5**), respectively. In alkaline pH, two degradation products, 2-hydroxy-*N*-(4-methyl-5-isoxazolyl)-1,4-naphthoquinone-4-imine (**3**) and 5-amino-4-methylisoxazole (**4**), were isolated. The pathway for degradation of **1** in acidic and neutral pH followed consecutive first-order kinetics since **2** undergoes hydrolysis giving 2-hydroxy-1,4-naphthoquinone (**6**) and 2-methylcyanoacetamide (**5**). No appreciable buffer effect on the degradation of **1** and **2** was observed for any of the buffer species in this study. The pH–rate profiles exhibited specific acid and specific basic catalysis for **1** and specific acid catalysis for **2**. The maximum stability for **1** and **2** occurred in the neutral pH region.

Previous studies about the biological behavior of new investigational isoxazolylnaphthoquinones have demonstrated that some compounds of the series show important biological properties.[1–4] These results and the importance for new drug development to know the chemical behavior of new active compounds, as well as that of their degradation products, encouraged us to extend our studies[5,6] of the conditions and mechanism by which these compounds are degraded.

In this paper the hydrolytic degradations of a diisoxazolylnaphthoquinone, 2-(4-methyl-5-isoxazolylamine)-*N*-(4-methyl-5-isoxazolyl)-1,4-naphthoquinone-4-imine (**1**), which was obtained by a method previously reported,[7] and its main degradation product, the 4-*N*-(4-methyl-5-isoxazolyl)-1,2-naphthoquinone (**2**), were investigated over a wide range of pH values and at different temperatures.

The quantitative analysis was performed by absorption and second-derivative[8,9] spectrometry. The latter method has proved to be a simple, rapid, and accurate method for the assay of **1** in presence of its degradation products at acidic and neutral pHs.

Experimental Section

Materials—All chemicals and reagents were analytical grade. The water used for buffers was doubly distilled and deionized through a Milli-Q water purification system (Millipore).

2-(4-Methyl-5-isoxazolylamine)-*N*-(4-methyl-5-isoxazolyl)-1,4-naphthoquinone-4-imine (1)—A solution of 0.001 mol (0.098 g) of 5-amino-4-methylisoxazole in 15 mL of 3M HCl was added to a solution of 0.0005 mol (0.130 g) of sodium-1,2-naphthoquinone-4-sulfonate in 15 mL of water. The reaction mixture was stirred for 30 min at room temperature and then boiled at reflux for 10 min. The insoluble red material formed was filtered, dried, and recrystallized from benzene:CCl_4, mp 183–184 °C; IR (KBr pellet): 3546, 1647, 1608, 1582, 1338, 1312, and 1266 cm^{-1}; ^1H NMR (DMSO-d_6 with tetramethylsilane as internal standard): δ 9.67 (s, 1H), 7.69–8.65 (m, 6H), 7.15 (s, 1H), 2.27 (s, 3H), and 2.15 (s, 3H) ppm; MS: *m/z* 334 (M^+).

4-*N*-(4-Methyl-5-isoxazolyl)-1,2-naphthoquinone (2)—Compound **2** was prepared by hydrolysis of **1** and then isolation by column chromatography on silica gel, using benzene as the elution media. Characterization of **2** was made by comparison of its ^1H NMR and IR spectra and melting points with those of an authentic sample which was prepared by reaction of sodium-1,2-naphthoquinone-4-sulfonate with 5-amino-4-methylisoxazole in 2M aqueous HCl at room temperature for 30 min. The insoluble product was recrystallized from benzene, mp 160–161 °C; IR (KBr pellet): 3559, 1687, 1623, 1587, and 1355 cm^{-1}; ^1H NMR (DMSO-d_6/TMS): δ 8.48 (2, 1H), 8.17–7.62 (m, 5H), 5.95 (s, 1H), and 2.61 (s, 3H) ppm; MS: *m/z* 254 (M^+).

Buffer Solutions—The following buffers were used for the general investigations: at pH < 1, HCl; at pH 1–2, KCl:HCl; at pH 2–8, McIlvaine buffer[10] (citric acid:Na_2HPO_4); at pH 8–10, NaOH:$NaHCO_3$; at pH 10–12, KCl:NaOH; and at pH > 12, NaOH. For the general acid-base catalysis, the following buffers were used: citric acid:Na_2HPO_4 (pH 2.30) and KCl:NaOH (pH 11.70) for **1**, and KCl:HCl (pH 2.28) for **2**. Constant ionic strengths (μ) of 0.5, 1.0, 1.5, and 2.0 were maintained for each buffer by adding an appropriate amount of NaCl. The relative amount of the ionic species in the buffers was calculated from the equations given by Laitinen.[11] The solutions were freshly prepared and the pHs were measured at 70 °C with an Orion model SA 520 pH-meter and SC-glass electrode.

Thin-Layer Chromatography—The TLC was conducted on precoated silica gel SIL G/UV_{254} sheets (Macherey-Nagel and Company) with benzene:chloroform:ethanol:sulphuric acid (14,4:20,0:2,6:0,5) as the developing solvents. Visualization was accomplished by fluorescence quenching under UV light (254 nm).

Apparatus—Ultraviolet spectrophotometric studies were carried out on a Shimadzu UV-260 spectrophotometer. The ^1H NMR spectra were recorded on a Varian T-60 spectrometer. The IR spectra were obtained on a Nicolet 5 SXC FT-IR. Melting points were determined on a Büchi 510 melting point apparatus and were uncorrected.

Kinetic Studies—A stock solution of the appropriate compound (1×10^{-4} M) was prepared in 95% ethanol. Aliquots were taken from the stock solution and diluted with various buffers to produce a final concentration of 1×10^{-5} M.

All sample solutions were filled into 10-mL flasks and then stored at 70 °C in a constant-temperature bath which was regulated by a thermostat (Haake F3) with ±0.1 °C precision. Samples were withdrawn at suitable time intervals, immediately cooled in an ice bath, and analyzed

Second-Derivative Ultraviolet Spectrophotometric Analysis—Solutions of **1** for standard curves were prepared by diluting the stock solution to obtain final concentrations in the range $2.01–3.35 \times 10^{-5}$ M. The second-derivative spectrum of absorbance (D_2A) with respect to wavelength at 273.1 nm was measured three times for each concentration. In all cases, plots of D_2A versus concentration at several pHs were lineal in the concentration range examined, showing agreement with Beer's law.

Results and Discussion

Characterization of the Degradation Products—The degradation products of **1** in basic pH were found to be 2-hydroxy-*N*-(4-methyl-5-isoxazolyl)-1,4-naphthoquinone-4-imine (**3**) and 5-amino-4-methylisoxazole (**4**) (Scheme I). Both products remained unchanged under the reaction conditions.

At acidic and neutral pHs, **1** gives 4-*N*-(4-methyl-5-isoxazolyl)-1,2-naphthoquinone (**2**) and 2-methylcyanoaceta-

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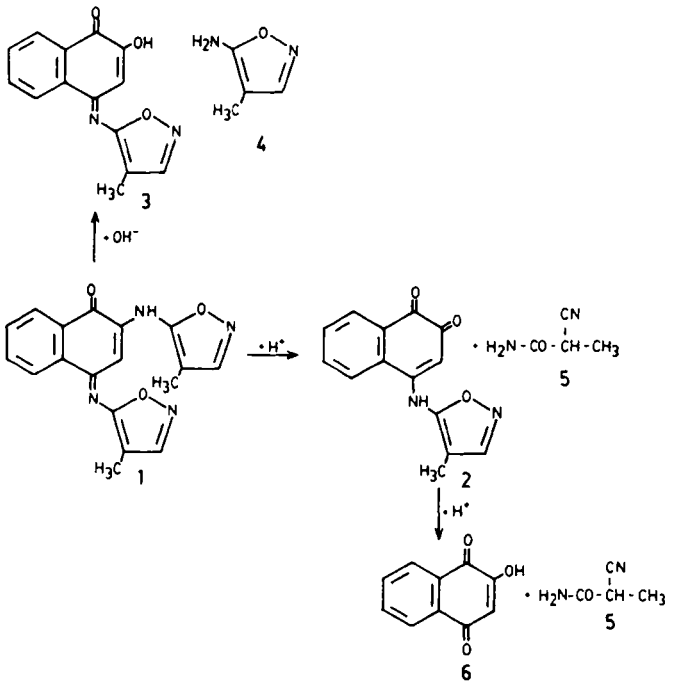


Scheme I

mide (5). Subsequently, 2 undergoes hydrolysis leading to 2-hydroxy-1,4-naphthoquinone (6) and 2-methylcyanoacetamide (5). The starting material (1) and its degradation products (2–6) were isolated and characterized by comparison of their R_f values and ^1H NMR, IR, and melting point data with those of authentic samples.[12,13]

Absorption (Zero-Order) and Second-Derivative Ultraviolet Spectrometry—The remaining concentration of 1, at basic pHs, was determined from the absorbance at 630 nm; this corresponds to a maximum in the absorption (zero-order) spectra of 1 without any interference from the signal of its degradation product (3) (Figure 1).

The absorption spectra (zero-order) of 1 and its degradation product 2, at acidic and neutral pHs, are shown in Figure 2A. The large band of the degradation product 2 interfered with the signal for 1, so determination of 1 by conventional absorption spectrometry would be difficult.

However, the second-derivative spectra for both compounds are quite different from each other (Figure 2B). While the starting material 1 possesses maxima and minima in the region 260–280 nm, 2 displays more or less a baseline spectrum in this region; therefore, the D_2A spectrum was used for the quantitative determination of residual 1 without interference of 2.

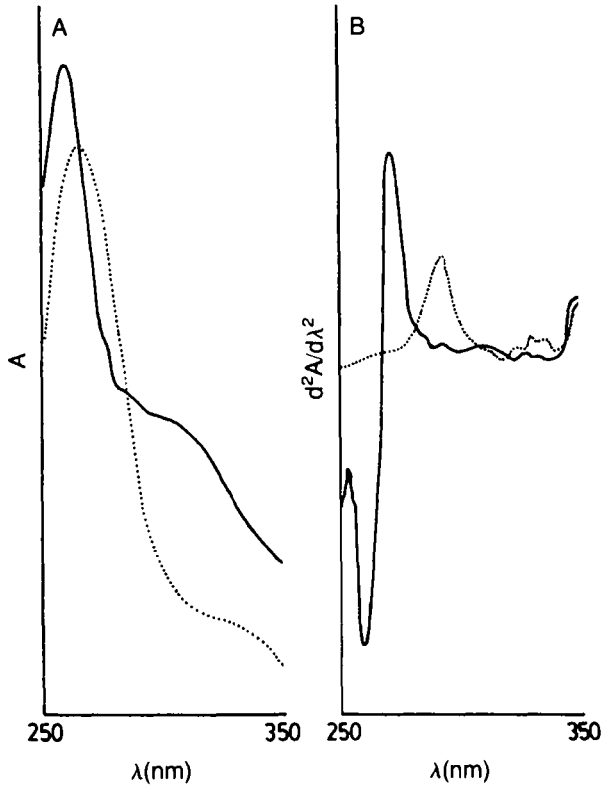


Figure 2—Zero-order absorption spectra (**A**) and second derivative spectra (**B**) of 1 and its degradation product 2 in solution of acidic and neutral pHs. Key: (——) 1; (····) 2.

For the kinetic studies of 2 in acidic media, the analyses were carried out measuring the absorbance at 444 nm, where only 2 absorbs (Figure 3).

Reaction Order and Rate Constants for Degradations of 1 and 2—The degradation of 1 was carried out at 70 °C and at a constant ionic strength (μ) of 0.5 over a pH range of 1.75 to

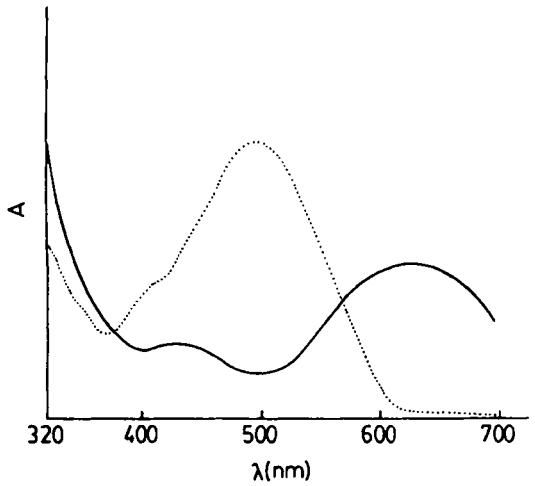


Figure 1—Absorption spectra of 1 and its degradation product 3 in solution of pH > 11. Key: (——) 1; (····) 3.

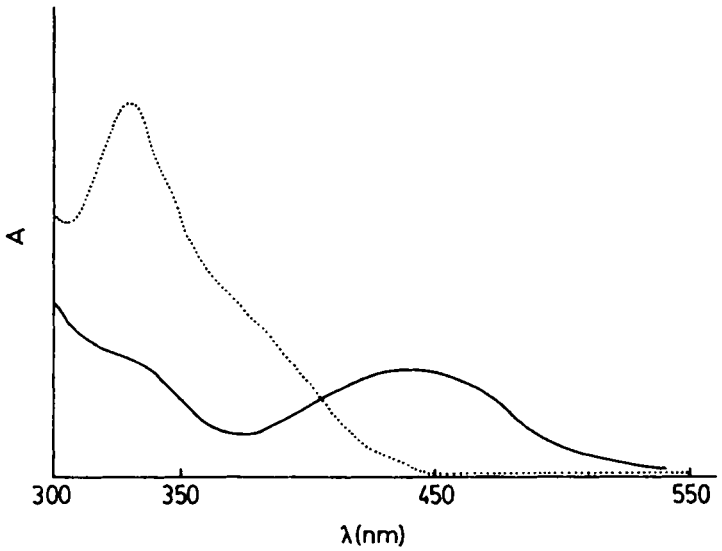


Figure 3—Absorption spectra of 2 and its degradation product 6. Key: (——) 2; (····) 6.

12.85 (Table I). At acidic pHs, the degradation of 1 takes place by a consecutive reaction according to Scheme I.

Since the value of k_{obs} for 1 (k_{obs} 1; 4.44 h^{-1} at pH 1.75) is greater than that for 2 (k_{obs} 2; 0.023 h^{-1} at pH 1.75), the two reactions can be considered to be independent (see Scheme II).

Plots of ln ($D_2A_0 - D_2A_t$) versus time (min) for 1 and ln ($A_0 - A_t$) versus time (min) for 2 (Figures 4 and 5, respectively) are linear ($r > 0.98$) under the experimental conditions.

The degradation reactions of 1 and 2 followed pseudo first-order kinetics, and their calculated rate constants along with buffer constituents are listed in Tables I and II, respectively.

pH–Rate Profile—The pH–rate profile for degradation of 1 at 70 °C and $\mu = 0.5$ is given in Figure 6. It exhibits a typical U-shape, with three important pH regions: one where a hydrogen ion-catalyzed reaction takes place (pH < 4.5); a pH-independent region (pH 4.5 to 8.5); and a region where the reaction was hydroxide ion catalyzed (pH > 8.5). Since slopes of the straight line portions of the profile are close to unity (pH < 4.5 and pH > 8.5), these two regions are associated with specific acid and specific base catalysis that obey the following general law:

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

where the rate constants k_H, k_o, and k_{OH} are the coefficients for the hydronium ion, water (or spontaneous reaction), and hydroxide ion-catalyzed degradation, respectively. The calculated values for k_H and k_{OH} were 31.5 and 20.4 h^{-1}, respectively.

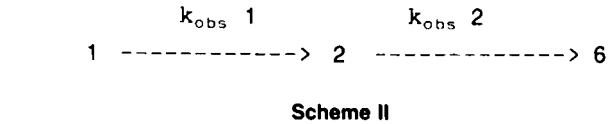
The inflection points observed in the pH–rate profile near pH 2.50 (pK_a 2.20) and pH 11.00 (pK_a 11.20) indicate that the dissociation equilibria of 1 influenced the degradation rates. At pHs from 4.5 to 8.5, 1 was in its neutral form, and this species was found to be more resistant to hydrolysis than the positively or negatively charged species.

The pH–rate profile for degradation of 2 is given in Figure 7. The profile shows that the observed first-order rate constant (k_{obs}) for degradation of 2 rapidly decreases with increasing pH from 0.0 to 2.5, with an inflection point near pH 1.00 (pK_a 0.70). From pH 3 to 7, the rate is independent of pH changes and 2 exists in its neutral form.

Table I—Rate Constants of 1 at Varying pH Values[a]

pH	Buffer Composition	$k_{obs} \times 10$, h^{-1}	$t_{1/2} \times 10$, h
1.75	HCl:KCl	44.4	1.56
1.95	"	31.4	2.21
2.10	Citric Acid:Na_2HPO_4	16.0	4.34
2.30	"	13.3	5.21
2.55	"	6.42	10.8
2.70	"	3.42	20.3
2.85	"	3.04	22.8
2.90	"	2.52	27.6
3.02	"	1.96	35.3
3.15	"	1.15	46.2
3.35	"	0.780	88.9
3.55	"	0.626	111.0
3.75	"	0.374	186.0
4.85	"	0.0523	1325.0
7.35	"	0.0580	1195.0
8.60	NaOH:$NaHCO_3$	0.0607	1142.0
9.65	"	0.741	93.5
10.70	KCl:NaOH	6.18	11.2
10.90	"	6.36	10.9
11.70	"	8.88	7.81
12.15	"	10.2	6.78
12.65	NaOH	17.3	4.01
12.85	"	17.5	3.95

[a] Constant temperature (70 °C) and ionic strength ($\mu = 0.5$).



Scheme II

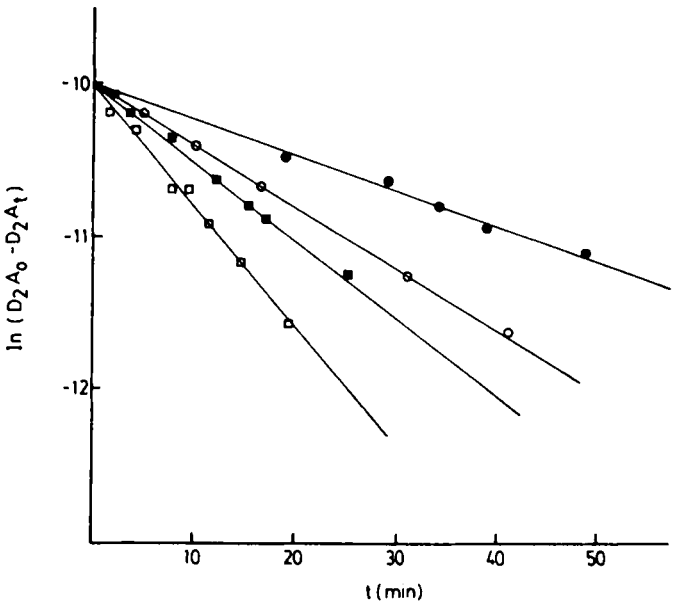


Figure 4—Plots of the observed pseudo first-order kinetic degradation of 1 in solution of different pHs and 70 °C ($\mu = 0.5$). Key: (●) pH 2.30; (○) pH 2.10; (■) pH 1.95; (□) pH 0.30.

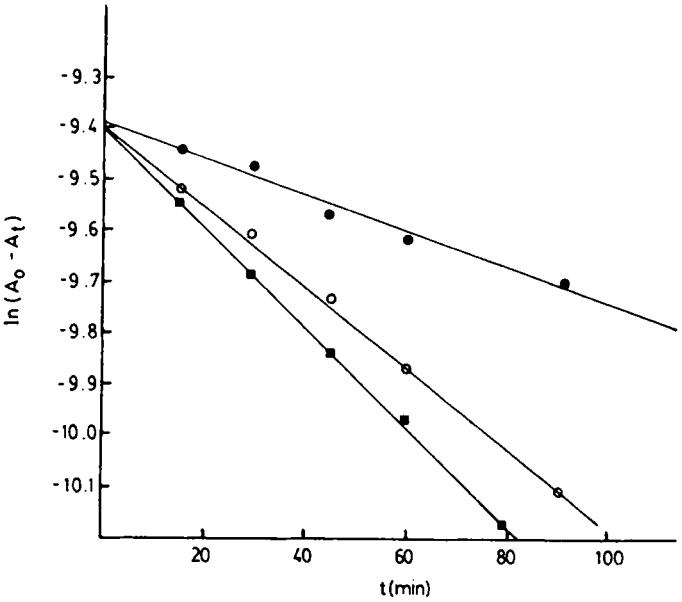


Figure 5—Plots of the observed pseudo first-order kinetic degradation of 2 in solution at different pHs and 70 °C ($\mu = 0.5$). Key: (●) pH 0.70; (○) pH 0.40; (■) pH 0.30.

The existence of specific acid catalysis is indicated by the slope close to unity in the region below pH 3 in the pH–rate profile, with a calculated k_H value of 1.05 h^{-1}.

Catalytic Effects of Buffers and Ionic Strength on the Degradation of 1 and 2—The catalytic effect of buffer components used in the kinetic studies was determined at constant pH, ionic strength ($\mu = 0.5$), temperature, and 1 and 2 concentration, varying only the buffer concentration. No

Table II—Rate Constants of 2 at Varying pH Values[a]

pH	Buffer Composition	$k_{obs} \times 10^2$, h^{-1}	$t_{1/2}$, h
0.30	HCl	59.3	1.17
0.40	"	47.3	1.46
0.70	"	20.9	3.31
1.18	HCl:KCl	10.6	6.55
1.40	"	4.26	16.3
1.55	"	3.52	19.7
1.74	"	2.31	30.0
2.07	"	1.49	46.6
2.28	Citric Acid:Na_2HPO_4	0.682	102.0
2.62	"	0.478	145.0
2.82	"	0.497	140.0
6.85	"	0.316	219.0

[a] Constant temperature (70 °C) and ionic strength (μ = 0.5).

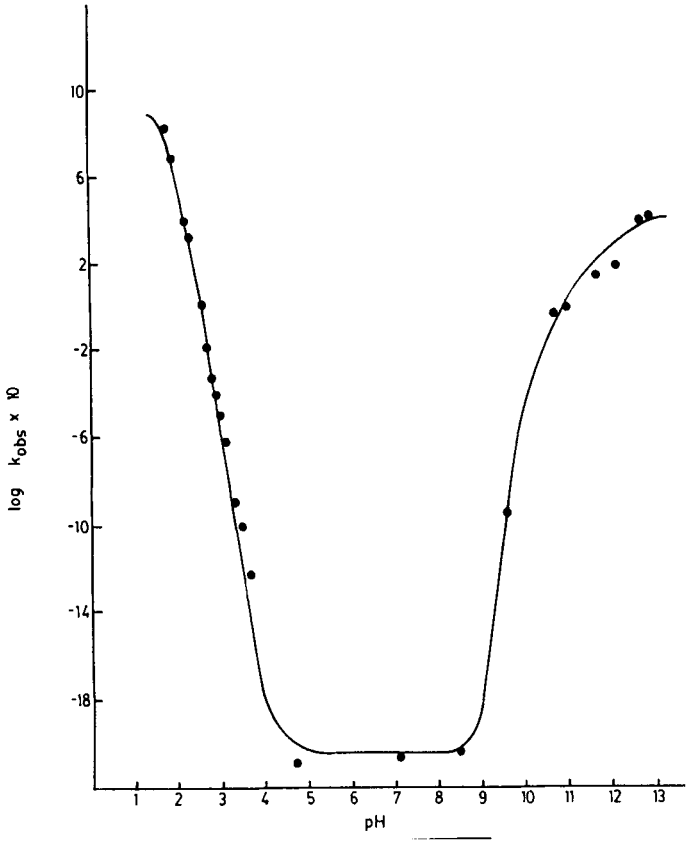


Figure 6—pH–Rate profile of the degradation of **1** at 70 °C and μ = 0.5.

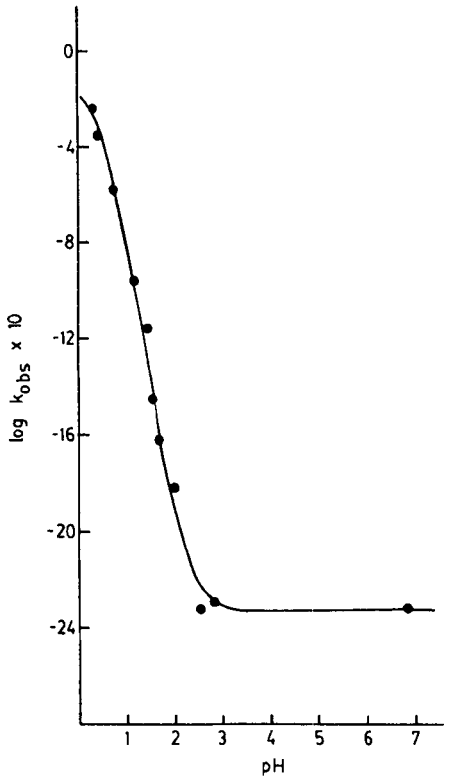


Figure 7—pH–Rate profile of the degradation of **2** at 70 °C and μ = 0.5.

Table III—Effect of Temperature on the Rate Constants of 1 and 2

Compound[a]	pH	Temperature, °C	$k_{obs} \times 10$, h^{-1}	$t_{1/2}$, h
1	2.30	25	0.194	35.7
		60	4.91	1.41
		70	13.3	0.521
		80	26.2	0.265
		90	31.4	0.221
2	0.30	25	0.111	62.4
		60	2.30	3.01
		70	5.93	1.17
		80	10.8	0.642
		90	20.2	0.343

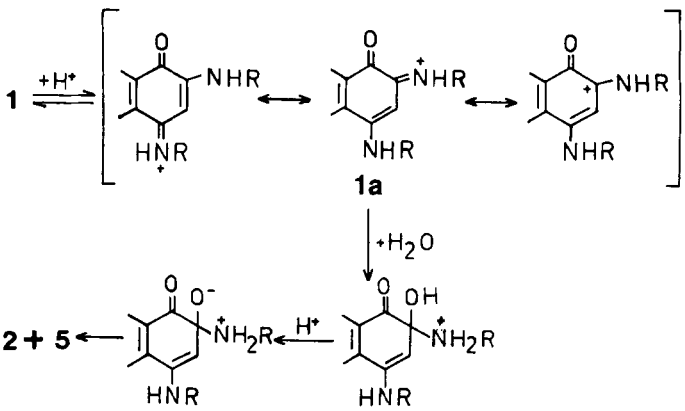
[a] E_a = 18.2 ± 0.4 kcal/mol for **1**; E_a = 17.1 ± 0.6 kcal/mol for **2**.

appreciable effect on the degradation of **1** and **2** was observed for any of the buffer species used in this study.

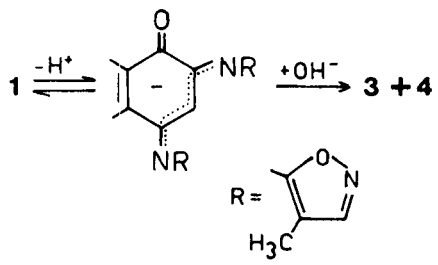
The effect of the ionic strength on the hydrolysis of **1** and **2** was determined by keeping the pH, buffer concentration, and temperature constant, and varying only the ionic strength by addition of different amounts of NaCl. No kinetic salt effect was seen for the degradation of the two compounds studied.

Temperature Effect on Reaction Rates of 1 and 2—The temperature effect on the hydrolytic reactions of **1** and **2** was determined by measuring the pseudo first-order rate constants at temperatures ranging from 60 to 90 °C. The calculated parameters, using Arrhenius plots, and the extrapolated first-order rate constant at 25 °C are given in Table III.

Possible Degradation Pathway of 1—The acid catalytic degradation of **1** can be explained according to Scheme III. As was observed for other similar compounds,[5] **1** undergoes hydrolysis through protonation on the N atom, leading to the iminium ion **1a**. Then, water addition on the iminium ion and subsequent deamination and isoxazole ring opening yields **2** and **5**; these compounds were observed experimentally. Since no other intermediate product was identified in the reaction



Scheme III



Scheme IV

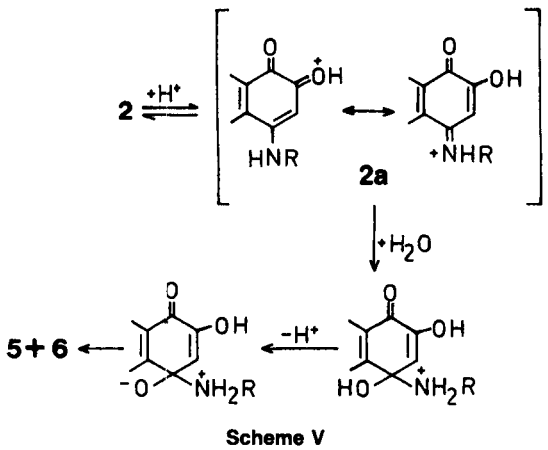
course by TLC, it was assumed that the rate-limiting step is the attack of the water on the protonated imine **1a**.

The postulated degradative reaction for **1** in basic solutions is shown in Scheme IV. According to previous reports on naphthoquinoneimines,[5,14–16] the basic hydrolysis of **1** would take place by addition of the hydroxide ion on C_2, leading to the stable degradation compounds **3** and **4** as a consequence of the C—N bond cleavage.

Possible Degradation Pathway of 2—Figure 7 shows that **2** undergoes specific acid-catalyzed degradation below pH 3.00. According to our previous studies[6] and the well-known hydrolysis of imines,[17–21] the acidic degradation of **2** involves cleavage of the C_4—N bond yielding **5** and **6** (Scheme V). A protonation occurs in the first step, leading to the structure **2a**. Then, by addition of water, the rate-limiting step, and the subsequent deamination and the isoxazole ring-opening process, **5** and **6** are formed.

Conclusions

The degradation reaction of **1** in aqueous solutions was catalyzed by acids and bases. The hydrolysis reaction occurred on carbon-2 of the naphthoquinone skeleton, with loss of the aminoisoxazolyl group. Its degradation product (**2**) in acidic medium underwent hydrolysis through a specific acid reaction, whereas its basic degradation product (**3**) showed good stability in solutions of high pH.



Scheme V

Although the degradation reactions followed a consecutive pseudo first-order kinetics, the significant differences between the values of $k_{obs}1$ and $k_{obs}2$ (half-time of 0.521 and 102 h, respectively) at pH $\approx$ 2.30 allowed the reactions to be treated as if they were independent of each other.

The behavior of these quinoneimines is in good agreement with that previously observed in compounds of analogous structure.[5,6]

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