Isoxazoles V: Chemical Stability of Diisoxazolylnaphthoquinone in Aqueous Solution

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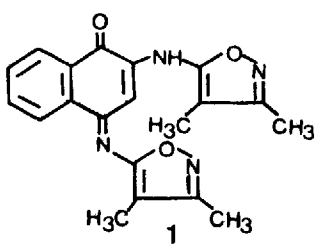
Abstract □ The hydrolytic degradation of 2-(3,4-dimethyl-5-isoxazolylamine)-*N*-(3,4-dimethyl-5-isoxazolyl)-1,4-naphthoquinone-4-imine (**1**) was investigated over a wide range of pH values and at different temperatures. The degradation rates were determined by reversed-phase HPLC and were observed to follow pseudo-first-order kinetics with respect to the concentration of **1**. The pH–rate profile was linear with slopes −1 and +1 in acid and alkaline pH, respectively, becoming pH independent in the region of maximum stability from pH 4.5 to 10.0. Neither primary salt effects nor buffer catalysis was observed due to the buffer species employed.

Previous work[1-4] about the mechanisms of acid-catalyzed degradation of some sulfonamide and amine derivatives of the isoxazole have demonstrated that the 5-*N*-substituted derivatives exhibit a particular lability of the ring as compared with the one observed for compounds *N*-substituted in positions 3 or 4. In connection with these studies we have determined[5,6] that aqueous solutions of isoxazole amines react with sodium-1,2-naphthoquinone-4-sulfonate to give highly colored compounds, some of which show important biological properties.[7,8]

Based on these findings and the well-known biological activities of many compounds of the naphthoquinone and isoxazole series, the importance of the conditions and mechanism by which these new compounds are degraded has greatly increased. This paper is concerned with the acidic, neutral, and alkaline hydrolysis of a diisoxazolylnaphthoquinone, 2-(3,4-dimethyl-5-isoxazolylamine)-*N*-(3,4-dimethyl-5-isoxazolyl)-1,4-naphthoquinone-4-imine (**1**), which was obtained by improving a previously reported method.[6] The quantitative analysis was performed by HPLC since the compound and its degradation products can be monitored simultaneously.[9]

Experimental Section

Materials—All chemicals and reagents were analytical grade. Methanol and ethanol were treated with 2,4-dinitrophenyl hydrazine according to the literature.[10] Water used for buffers and the mobile phase in HPLC was double distilled and deionized through a Milli-Q water purification system (Millipore). All the solvents used for HPLC were filtered through a 0.22-μm filter prior to use.



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2-(3,4-Dimethyl-5-isoxazolylamino)-*N*-(3,4-dimethyl-5-isoxazolyl)-1,4-naphthoquinone-4-imine (1)—A solution of 0.002 mol (0.224 g) of 5-amino-3,4-dimethylisoxazole in 40 mL of hydrochloric acid (3M) was added to a solution of 0.001 mol (0.260 g) of sodium-1,2-naphthoquinone-4-sulfonate in 10 mL of water. The reaction mixture was stirred for 30 min at room temperature and then boiled at reflux for 10 min. The insoluble brown material was filtered, dried, and recrystallized from benzene:CCl_4 (60% yield).

2-Hydroxy-*N*-(3,4-dimethyl-5-isoxazolyl)-1,4-naphthoquinone-4-imine (2) and 4-*N*-(3,4-Dimethyl-5-isoxazolyl)-1,2-naphthoquinone (4)—Compounds 2 and 4 were obtained and purified by a previously reported procedure.[6]

5-Amino-3,4-dimethylisoxazole (3)—Compound **3** was prepared according to a method previously described.[1] The ^1H NMR, IR, and mass spectra and the melting point of **1**, **2**, and **4** are in agreement with those published in the literature.[6]

Buffer Solutions—For the general investigations the buffers used were as follows: at pH < 1, HCl; at pH 1–2, KCl:HCl; at pH 2–8, McIlvaine buffer (citric acid:Na_2HPO_4)[11]; at pH 8–10, NaOH:$NaHCO_3$; at pH 10–12, KCl:NaOH; at pH > 12, NaOH. For the general acid-base catalysis, the buffers used were as follows: citric acid:Na_2HPO_4 (pH 2.95) and KCl:NaOH (pH 12.10).

Constant ionic strengths 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.[12] The solutions were freshly prepared and the pH values were measured at the experimental temperature by a research pH meter and SC-glass electrodes.

Kinetic Procedure—The quinone-diimine **1** was dissolved in ethanol up to a concentration of 1×10^{-4} M. This was used as the stock solution. For stability tests, the stock solution was diluted with buffer of the appropriate pH up to a concentration of 1×10^{-5} M. The solution was filled into a 10-mL flask, and then stored in a constant temperature bath which was regulated by a thermostat (Haake F_3) with ±0.1 °C precision. At appropriate intervals, the flasks were taken from the bath and cooled on an ice bath, and the solution was analyzed by HPLC.

High-Performance Liquid Chromatography Analysis—The HPLC system consisted of a Shimadzu Liquid Chromatograph equipped with an LC-2A pumping system and an SPD detector which was connected to an Altex CR Integrator. The separation of **1** from its degradation products was achieved at room temperature under the following conditions: the column (250 × 4 mm) was packed with Lichrosorb RP18, 10 μm (Hibar-Merck); the mobile phase was methanol:water (80:20); the flow rate was 2.0 mL/min; the injection volume was 10 μL and the detection was made at 310 nm.

Peak heights were measured and the concentrations were calculated from calibration curves. Standard solutions of **1**, **2**, and **4** were accurately prepared in ethanol. A series of dilutions covering the range 0.9–9.0 μg/mL was made to determine the sensitivity and linearity of the response. The calibration curve of peak heights against the concentration of **1** was linear.

The mass spectra were recorded on a Finnigan model 3300 F-100 quadrupole mass spectrometer. Data was collected and processed with an INCOS data system using a Nova III computer. The ^1H NMR spectra were recorded on a Varian T-60. Melting points were determined on a Büchi 510 melting point apparatus and were uncorrected.

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Results and Discussion

Identification of Degradation Products—The kinetics for the degradation of 1 was followed at various pH values by HPLC. Typical chromatograms are shown in Figure 1. The chromatogram at neutral pH is the same as that at acidic pH. The peak designated as 1 is the parent compound and those designated 2 and 4 are the hydrolysis degradation products in alkaline and acidic media, respectively. The identification of the last two compounds is based on a comparison of the HPLC retention times with those of authentic samples.

At basic pH, 1 hydrolyzes to 2-hydroxy-*N*-(3,4-dimethyl-5-isoxazolyl)-1,4-naphthoquinone-4-imine (2) and to 5-amino-3,4-dimethylisoxazole (3) according to Scheme I.

At acidic and neutral pH, 1 degrades to give 4-*N*-(3,4-dimethyl-5-isoxazolyl)-1,2-naphthoquinone (4), as the major product. The other products, 2-butanone (5), ammonia (6), hydroxylamine (7), and carbon dioxide (8), were found to be the acidic hydrolysis products[1] of 3 (Scheme I).

Reaction Order and Rate Constants—Plots of the natural log (ln) of residual concentrations of 1 versus time (Figure 2) exhibit good linearity ($r > 0.98$) for experiments at all pH levels studied. The disappearance of 1 followed pseudo-first-order kinetics at 70 °C and at constant ionic strength (μ = 0.5) over a pH range of 1.70 to 13.00.

The degradation rate constants of 1 were computed by the least-squares linear regression method. The calculated rate constants and the buffer systems employed are shown in Table I.

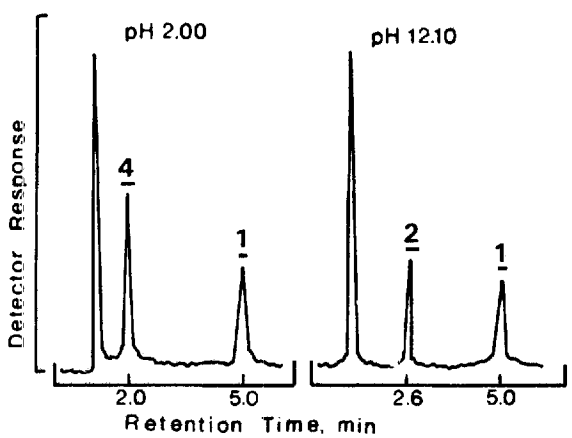
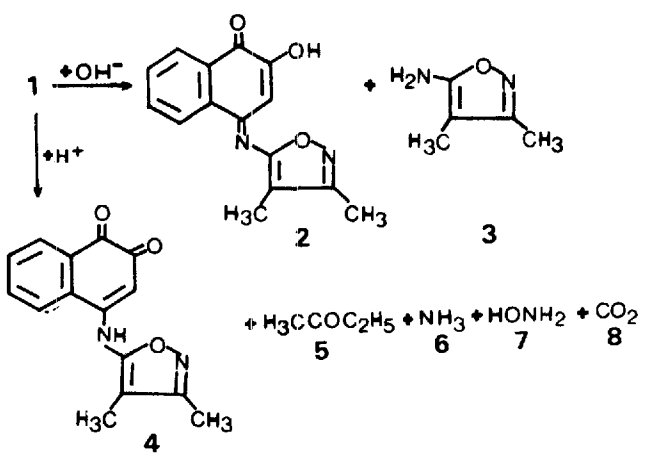


Figure 1—*High-pressure liquid chromatograms of the degradation of* 1 *(pH 2.00, resolution = 13.3; pH 12.10, resolution = 9.60).*



Scheme I

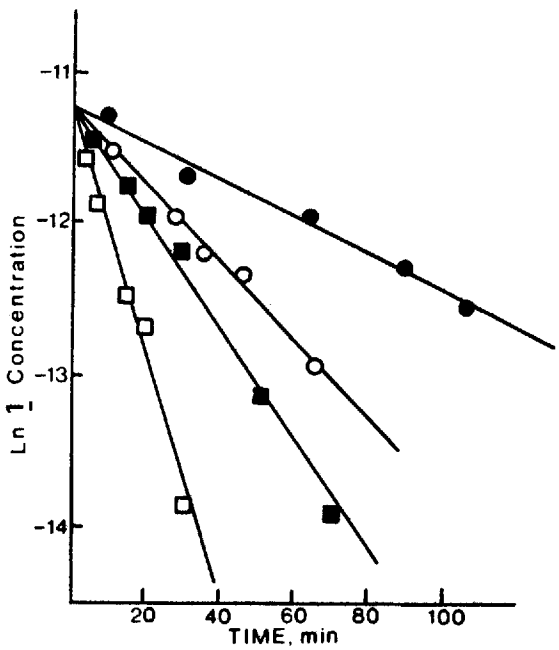


Figure 2—*Plots of the observed pseudo-first-order kinetic degradation of* 1 *in solution at different pH values and 70 °C (μ = 0.5). Key:* (●) *pH 2.95;* (○) *pH 2.50;* (■) *pH 2.35;* (□) *pH 2.25.*

Catalytic Effect of Buffer Systems—The catalytic effect of the buffer system used in the kinetic studies was determined at constant pH, temperature, ionic strength, and drug concentration of 1; only the buffer concentration varied. These experiments were done at pH 2.95 and pH 12.10. No appreciable effect on the degradation of 1 was observed for any of the buffer species used in this study (Table II).

Salt Effect—The salt effect on the hydrolysis of 1 was studied at constant pH, temperature, and drug concentration, but the ionic strength was varied with sodium chloride addition. Studies were conducted at pH 2.95 and 12.10 for ionic strength values ranging from 0.5 to 2.0. The data obtained are given in Table III. No kinetic salt effects were observed, indicating that at least one of the reacting molecules is uncharged.

pH–Rate Profile—The pH dependence of the overall first-order rate constant for the degradation of 1 at 70 °C and an ionic strength of 0.5 is shown in Figure 3. The pH–rate profile exhibits an 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 10.0); and a region where the reaction was hydroxide ion-catalyzed (pH >10.0). Since slopes of the straight line portions of the profile are close to unity (pH <4.5 and pH >10.0), 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 a spontaneous reaction), and hydroxide ion catalyzed degradation, respectively. Scheme II describes the hydrolysis of 1 according to eq 1.

The inflection points observed in the pH-rate profile near pH 2.00 and 11.00 indicate that the dissociation equilibria of 1 (pK_a = 2.30 and pK_a = 11.00) influenced the degradation rates. These apparent pK_a values were calculated by the Connors method.[13,14]

Temperature Dependence—The degradation dependence of 1 on temperature was determined by measuring the rate of

Table I—Observed Rate Constants at 70 °C and $\mu = 0.5$

pH	Buffer		$k_{obs} \times 10^2$, h^{-1}	$t_{1/2} \times 10^2$, h
	Composition	Concentration, M		
1.70	HCl:KCl	2.10×10^{-2}–5.00×10^{-2}	633.8	10.9
1.90	HCl:KCl	1.90×10^{-2}–5.00×10^{-2}	573.3	12.1
2.00	Citric acid:Na_2HPO_4	2.09×10^{-1}–2.80×10^{-3}	484.9	14.3
2.25	Citric acid:Na_2HPO_4	1.96×10^{-1}–3.90×10^{-3}	493.5	14.1
2.35	Citric acid:Na_2HPO_4	1.88×10^{-1}–1.24×10^{-3}	290.2	23.9
2.50	Citric acid:Na_2HPO_4	1.78×10^{-1}–2.19×10^{-2}	230.2	30.1
2.95	Citric acid:Na_2HPO_4	1.59×10^{-1}–4.13×10^{-2}	88.16	182
3.35	Citric acid:Na_2HPO_4	1.43×10^{-1}–5.73×10^{-2}	22.29	311
3.70	Citric acid:Na_2HPO_4	1.23×10^{-1}–7.75×10^{-2}	17.88	388
4.50	Citric acid:Na_2HPO_4	1.07×10^{-1}–9.41×10^{-2}	0.96	7220
5.60	Citric acid:Na_2HPO_4	8.38×10^{-2}–1.16×10^{-1}	1.08	6418
7.00	Citric acid:Na_2HPO_4	3.52×10^{-2}–1.66×10^{-1}	1.02	6795
8.10	NaOH:$NaHCO_3$	4.70×10^{-3}–2.50×10^{-2}	1.08	6418
9.80	NaOH:$NaHCO_3$	1.07×10^{-2}–2.50×10^{-2}	2.34	2962
12.10	KCl:NaOH	5.00×10^{-2}–1.20×10^{-2}	7.16	968
13.00	NaOH	1.00×10^{-1}	8.46	819

Table II—General Acid-Base Catalysis of Degradation of 1 in Solutions at pH 2.95 and 12.00 and 70 °C

pH	Buffer Composition	Buffer Concentration	$k_{obs} \times 10^2$, h^{-1}
2.95	Citric acid:Na_2HPO_4	$1 \times C_0$	88.16
		$2 \times C_0$	89.01
		$3 \times C_0$	87.60
		$4 \times C_0$	88.52
12.10	KCl:NaOH	$1 \times C_0$	7.16
		$2 \times C_0$	7.13
		$3 \times C_0$	7.14
		$4 \times C_0$	7.18

Table III—Salt Effect on Degradation of 1 in Solutions at pH 2.95 and 12.00 and 70 °C

pH	Buffer Composition	μ	$k_{obs} \times 10^2$, h^{-1}
2.95	Citric acid:Na_2HPO_4	0.5	88.16
		1.0	87.62
		1.5	87.91
		2.0	88.55
12.00	KCl:HCl	0.5	7.16
		1.0	7.10
		1.5	7.17
		2.0	7.20

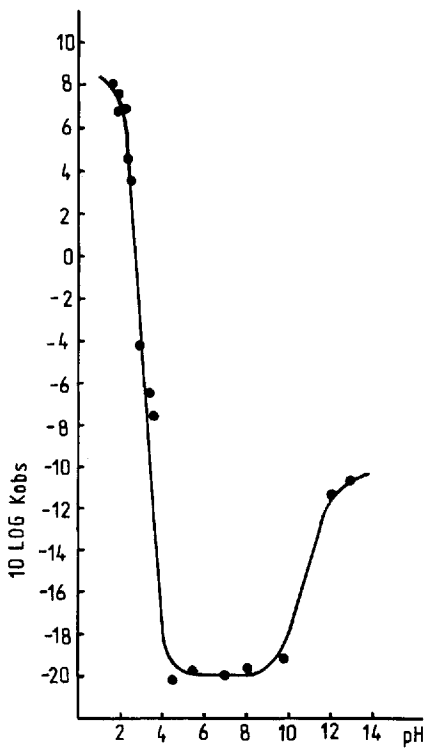


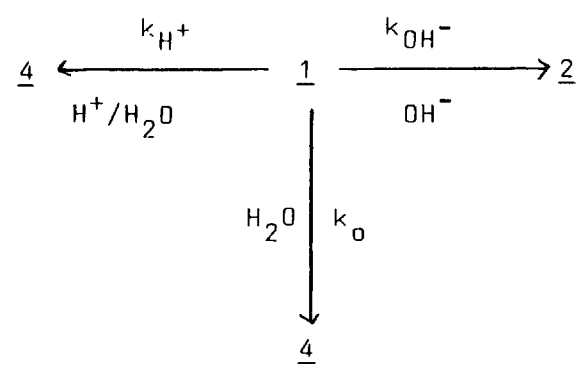
Figure 3—*pH–rate profile of the degradation of a solution of* **1** *at 70 °C and $\mu = 0.5$.*

decomposition at 60, 70, 80, and 90 ± 0.1 °C at pH 2.25 and 7.00 and ionic strength of 0.5. The values of k_{obs}, Ea, and log *A* are given in Table IV, and the corresponding Arrhenius plots are shown in Figure 4.

Possible Degradation Mechanism—Figure 3 shows that **1** undergoes specific acid-catalyzed degradation below pH 4.5. This process follows in general the typical mechanism for the hydrolysis of imines,[13–15] which involves addition of water and elimination of ketones and nitrogen moieties.

In the hydrolysis of **1**, the mechanism implies a preequilibrium between the substrate and the protonated substrate,[16] with subsequent rearrangement leading to iminium ions (**9**). Therefore, a series of resonance structures can be written (Scheme III). Some of these structures are similar to those proposed by Bullock[17] for other naphthoquinoneimines.

Taking into account all the structures proposed, **9c**, which bears a positive charge on carbon-2, would significantly contribute to the water addition on the iminium ion.[13,15,18–20]



Scheme II

Table IV—Apparent Heat of Activation of Degradation of 1 in Solutions at Constant pH and Ionic Strength μ = 0.5

Buffer pH	Rate Constant × 10^3, h^{-1}					E_a, kcal/mol (SD)
	25 °C	60 °C	70 °C	80 °C	90 °C	
2.25	1.75	26.9	49.4	155	238	17.3 (0.5)
7.00	0.0082	3.77	10.2	23.2	27.6	19.8 (0.5)

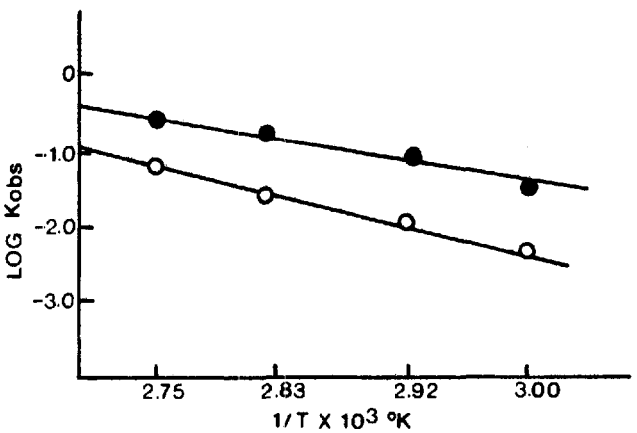
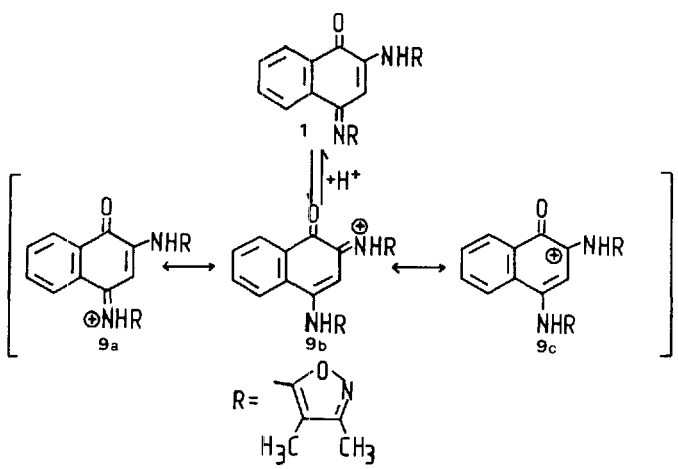


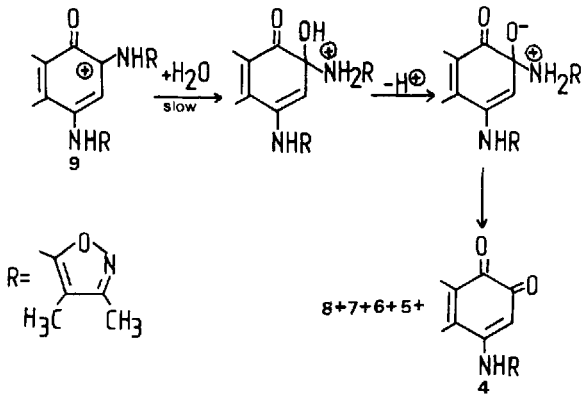
Figure 4—*Arrhenius-type plots showing temperature dependence of reaction rates of hydrolysis of* **1** *at two pH values: Key:* (○) *pH 7.00;* (●) *pH 2.25.*

The subsequent deamination and isoxazole ring-opening process and the proton transfer step yields the hydrolytic decomposition products 4, 5, 6, 7, and 8 which were experimentally observed (Scheme IV).

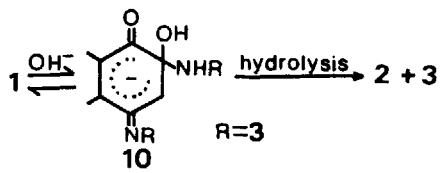
The degradation of 1 in alkaline medium takes place through a specific base-catalysis process (Figure 3). According to the products obtained (2 and 3) and from the hydrolysis of naphthoquinoneimines,[21-23] the degradation of 1 would occur through the formation of an intermediate like 10 by addition of the hydroxide ion to the carbon-2 (Scheme V). The rate-determining step in this case is the cleavage of the C—N bond since only one intermediate was observed in the reaction medium.



Scheme III



Scheme IV



Scheme V

Conclusions

Compound 1 is a disubstituted naphthoquinone which undergoes irreversible acid-base hydrolysis on the carbon-2 of the naphthoquinone skeleton with the aminoisoxazolyl substituent as the leaving group. The pH–rate profile reveals specific acid-base catalysis with a pH-independent plateau from pH 4.50 to 10.00.

In general, the quinone-diimine is more stable in alkaline solutions than in acidic ones, and according to our experiments, it can be predicted that at 25 °C and at pH 7.00, 1 exhibits a t_{90} of 54 d.

References and Notes

1. Manzo, R. H.; Bertorello, M. M. de *J. Pharm. Sci.* **1973**, *62*, 152.
2. Manzo, R. H.; Bertorello, M. M. de *J. Pharm. Sci.* **1973**, *62*, 154.
3. Manzo, R. H.; Bertorello, M. M. de *J. Org. Chem.* **1978**, *43*, 1173.
4. Ahumada, A. A.; Manzo, R. H.; Bertorello, M. M. de *Anales Asoc. Quím. Argentina* **1983**, *71*, 139–147.
5. Manzo, R. H.; Catania, P.; Bertorello, M. M. de *Anal. Chem.* **1974**, *46*, 1141.
6. Fernández, A. E.; Bertorello, M. M. de; Manzo, R. H. *Anales Asoc. Quím. Argentina* **1982**, *70*, 49.
7. Schwarcz, M. N.; Stoppani, A. O. Congreso Argentino de Protozoología, Córdoba, Oct. 1984, Abstr. 18.
8. Amuchástegui, P.; Basso, B.; Moretti, E.; Sperandeo, N.; Bertorello, M. M. de; Briñón, M. C. Congreso Sociedad de Biología de Córdoba, May 1987, Abstr. 42.
9. Fernández, A. E.; Bertorello, M. M. de; Longhi, M. R. *J. Liq. Chromatogr.* **1984**, *7(11)*, 2203–2217.
10. Perri, D. D.; Armarego, W.; Perrin, D. R. *Purification of Laboratory Chemicals;* Pergamon: Oxford, U.K.; 1982; 321.
11. McIlvaine, T. C. *J. Biochem.* **1921**, *49*, 183.
12. Laitinen, H. A. *Chemical Analysis*; McGraw-Hill: New York, 1960; p 35.
13. Connors, K. A. *Reaction Mechanisms in Organic Analytical Chemistry;* John Wiley & Sons: New York, 1973; pp 81, 470.
14. Connors, K. A. *J. Parent. Sci. Technol.* **1981**, *35(4)*, 186.
15. March, J. *Advanced Organic Chemistry,* 3rd ed.; John Wiley & Sons: New York, 1985; pp 784–786.

16. Sosa, V. E.; Longhi, M. R.; Bertorello, M. M. de; Briñón, M. C., unpublished results.
17. Bullock, F. J.; Tweedie, J. F.; McRitchie, D. D. *J. Chem. Soc. (c)* **1969**, *13*, 1799–1803.
18. Böhme, H.; Viehe, H. G. *Advanced Organic Chemistry;* John Wiley & Sons: New York, 1976; Vol. 9, p 108.
19. Hauser, C. R.; Lednicer, D. *J. Org. Chem.* **1959**, *24*, 46–49.
20. Sayer, J. M; Comlon, P. *J. Am. Chem. Soc.* **1980**, *102(10)*, 3592.
21. Tong, L. K.; Glessmann, M. C.; Bent, R. L. *J. Am. Chem. Soc.* **1960**, *82*, 1988–1996.
22. Reeves, R.; Tong, L. *J. Am. Chem. Soc.* **1962**, *84*, 2050–2057.
23. Reeves, R.; Tong, L. *J. Org. Chem.* **1965**, *30*, 237–242.

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