Kinetics and Mechanism of the Hydrolysis of a 2-Substituted Imidazoline, Cibenzoline (Cifenline)

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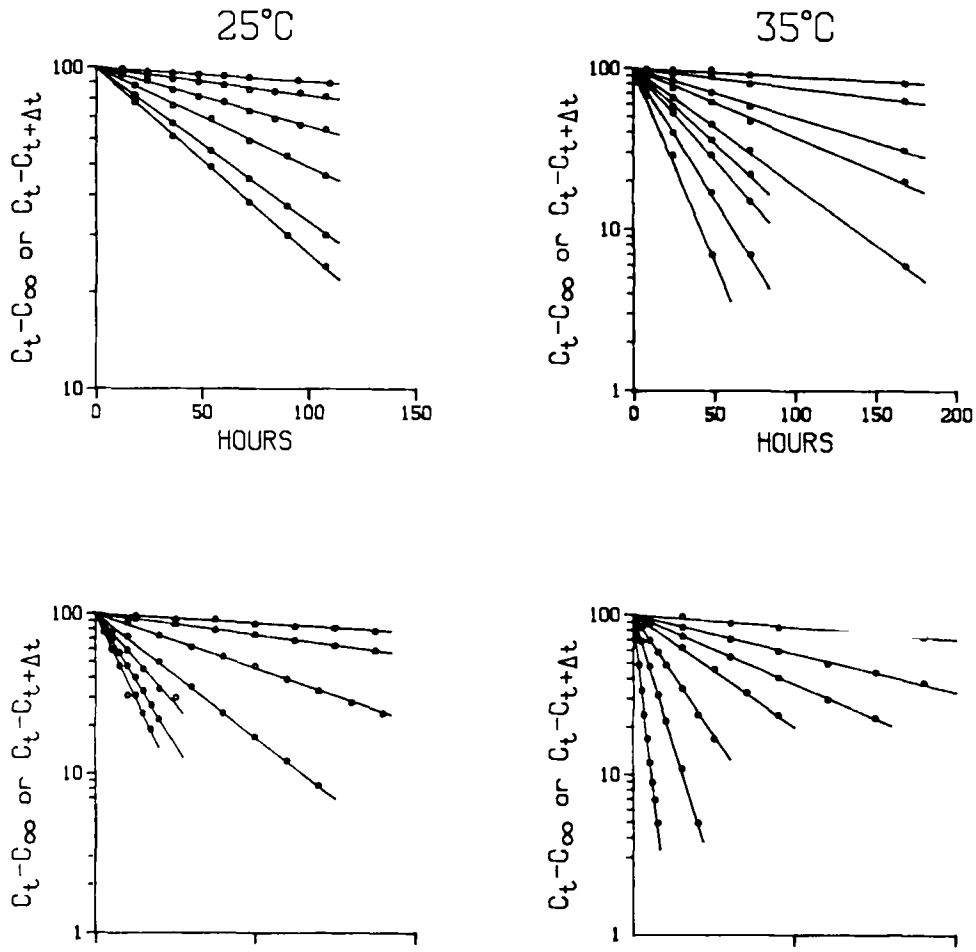
Abstract □ The results of a kinetic and mechanistic study of the hydrolysis of a new antiarrhythmic agent, cibenzoline, are reported. The reaction is subject to specific base catalysis which proceeds via the protonated cibenzolinium ion. No evidence for the existence of a "pseudobase"-type intermediate could be found. The results support only one of two different mechanisms which have been proposed previously for the hydrolysis of this class of compounds.

Cibenzoline [2-(2,2-diphenylcyclopropyl)-4,5-dihydro-1*H*-imidazol, 1][1,2] is currently under development as a new antiarrhythmic agent, and its stability is therefore a subject of interest. The generic name of this compound has recently been changed from cibenzoline to cifenline.[3] We report here the results of a kinetic and mechanistic analysis of the major cause of instability of cibenzoline, that is, hydrolysis to *N*-(2-aminoethyl)-2,2-diphenylcyclopropanecarboxamine (2).

Experimental Section

Materials—Cibenzoline succinate [2-(2,2-diphenylcyclopropyl)-4,5-dihydro-1*H*-imidazole; 1] and *N*-(2-aminoethyl)-2,2-diphenylcyclopropanecarboxamine (2) were used as received (Hoffman-La Roche Inc., Nutley, NJ). All other chemicals were of reagent grade. Distilled, deionized water was used throughout.

Chromatography—The HPLC system consisted of a solvent delivery system (model M-6000A; Waters Associates, Milford, MA), a sample processor (WISP model 710B; Waters), a strong cation exchange column (Partisil 10 SCX, 25 cm × 4.6 mm; Whatman, Clifton, NJ), and a detector (model UV-50; Varian Associates, Palo Alto, CA). Absorbance was measured at 230 nm. Recording and analysis of data were performed by the Computer Automated Labo-



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ratory System, CIS Inc., Waldwick, NJ. The detector response to 1 and 2 was linear over the concentration range of interest. The injection volume was 100 μL and samples were eluted with a mobile phase of 0.1 M KH_2PO_4 (pH 2.5) and methanol in the ratio 80:20 (v/v) at a flow rate of 1.25 mL/min. Retention times for 1 and 2 were 10 and 5 min, respectively.

Additional Equipment—The UV data were obtained with a Varian-Cary 219 spectrophotometer equipped with a thermostatable cell holder, cell programmer, and timer. The NMR spectra were recorded on a Varian XL-200 spectrophotometer operating at 50.309 MHz for carbon-13. Measurements of the pH of kinetic runs were made at the temperature of the run using an Orion model 0103 combination electrode and an Orion model 901 pH meter (standardized at the appropriate temperature using commercially available buffers supplied with temperature correction factors). Hydroxide ion concentrations were calculated from the measured pH values, the pK_w values (Table I) and the equation $[OH^-] = 10^{-(pK_w - pH)}$. Potentiometric titrations were performed on a Radiometer system consisting of a PHM82 pH meter, a TTA80 titrator and titration assembly, an ABU80 autoburette, and an REA160 titrigraph module.

Kinetics—Hydrolysis of 1 was studied using both UV and HPLC in the following ways. For UV analyses, reactions were initiated by the addition of 125 μL of a freshly prepared stock solution of 1 (~12 mg in 25 mL of 1 M KCl) to 2 mL of buffer or KOH solution in 1-cm teflon-stoppered quartz cuvettes thermostated at 25, 50, or 80 °C. After allowing time for temperature equilibration, reactions were monitored by recording the decrease in absorbance at 235 nm (the wavelength corresponding to the maximum molar extinction coefficient ratio of 1 to 2). For HPLC analyses, reactions were initiated by the addition of 1 mL of a stock solution of 1 (50 mg dissolved in 10 mL of methanol + 2 mL of 0.5% H_3PO_4, made up to 100 mL with water) to 25 mL of buffer or NaOH solution at 35 °C. At selected time intervals, 3-mL aliquots were withdrawn and the reaction was quenched by the addition of 1 mL of 0.33 M H_3PO_4. These solutions were assayed directly for 1 and 2 by HPLC. Rate constants were obtained either from semilogarithmic plots of C_t or $C_t - C_\infty$ versus time or by the Guggenheim method for slow reactions. Typical plots are shown in Figure 1, and observed rate constants are shown in Table II. All reactions were carried out at an ionic strength of 1.0 (KCl).

Determination of the pK_a of the Conjugate Acid of Cibenzoline by Potentiometric Titration—Solutions of 1 (0.01 M, ionic strength of 1.0, KCl) containing various proportions of methanol sufficient to keep the cibenzoline free base in solution during the titration were titrated with 0.1 M KOH (ionic strength = 1.0) at room temperature. The apparent pH values at 50% neutralization of the conjugate acid are shown in Table III. Linear extrapolation to 0% methanol gives a value of 10.70 ± 0.02 for the pK_a in water at an ionic strength of 1.0.

Table I—Kinetic Data for the Hydrolysis of Cibenzoline

Temperature, °C	pK_w[a]	Kinetic pK_a	k_o, h^{-1}	k_{OH^-}, $M^{-1}\cdot h^{-1}$
25	14.00	10.90	—	15.5
35	13.69	10.50	—	78.0
50	13.28	10.25	—	275
80	12.62	9.65	0.01	2100

[a] The pK_w data was interpolated from published data (*CRC Handbook of Chemistry and Physics*, 63rd ed.; CRC: Boca Raton, FL, 1982–1983; p D-173).

Results and Discussion

The hydrolysis of 1 between pH 7 and 13 at 25, 35, 50, and 80 °C follows pseudo first-order kinetics, and HPLC analysis showed that the rates of disappearance of 1 and appearance of 2 were identical. An authentic sample of 2 co-eluted with, and had the same spectral characteristics as, the degradation product formed in situ from 1. Catalysis by buffers (phosphate, borate, or carbonate up to 0.1 M) or by methoxyethylamine (0.055 M) or methoxylamine (0.034 M), was not detected. Hydrolysis of an authentic sample of 2 did not occur. The kinetic data are shown in the form of pH–rate profiles at different temperatures in Figure 2.

The data are consistent with a mechanism involving reaction of the protonated form of 1 ($1H^+$) with water (k_o) and hydroxide ion (k_{OH^-}). Contribution from the water reaction

Table II—Observed Rate Constants (k_{obs}) for the Hydrolysis of Cibenzoline

Temperature, °C	Buffer[a]	pH	k_{obs}, h^{-1}	Temperature, °C	Buffer[a]	pH	k_{obs}, h^{-1}
25	Carbonate	9.70	7.3×10^{-4}	35	Borate	8.84	1.1×10^{-3}
		9.73	1.0×10^{-3}		Carbonate	9.29	2.8×10^{-3}
		9.91	1.0×10^{-3}			9.74	6.8×10^{-3}
		10.18	1.9×10^{-3}			9.92	9.5×10^{-3}
		10.24	1.9×10^{-3}			10.09	1.6×10^{-2}
	Phosphate	10.57	4.2×10^{-3}			10.30	2.1×10^{-2}
		10.69	4.1×10^{-3}		Phosphate	10.45	2.6×10^{-2}
		11.04	7.1×10^{-3}			11.01	3.6×10^{-2}
		11.08	6.9×10^{-3}			11.40	4.4×10^{-2}
		11.39	8.9×10^{-3}			11.88	4.9×10^{-2}
		11.70	1.1×10^{-2}		Hydroxide	12.31	4.8×10^{-2}
	Hydroxide	11.81	1.1×10^{-2}			12.84	5.4×10^{-2}
		12.55	1.2×10^{-2}				
		12.71	1.3×10^{-2}	80	Phosphate	7.23	1.9×10^{-2}
		12.84	1.2×10^{-2}			7.42	2.3×10^{-2}
					Borate	7.99	5.8×10^{-2}
50	Borate	8.63	6.7×10^{-3}			8.22	1.0×10^{-1}
	Carbonate	9.13	1.5×10^{-2}			8.45	1.6×10^{-1}
		9.53	3.9×10^{-2}		Carbonate	8.97	3.5×10^{-1}
		9.54	4.0×10^{-2}			9.37	7.4×10^{-1}
		9.98	9.0×10^{-2}			9.77	9.9×10^{-1}
		10.00	9.6×10^{-2}		Phosphate	9.82	1.4
	Phosphate	10.25	1.3×10^{-1}			10.04	1.8
		10.60	1.9×10^{-1}			10.04	1.7
		10.84	2.3×10^{-1}			10.48	2.2
		10.92	2.1×10^{-1}		Hydroxide	11.16	2.0
		11.18	2.3×10^{-1}			11.43	2.0
		11.25	2.3×10^{-1}				
	Hydroxide	11.88	2.4×10^{-1}				
		12.23	2.4×10^{-1}				

[a] Ionic strength is 1; 0.1 M.

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Table III—Apparent pH Values at 50% Neutralization of Cibenzolinium Ion in Aqueous Methanol Solutions

Percent Methanol	Apparent pH at 50% Neutralization
20	10.73
30	10.74
40	10.76

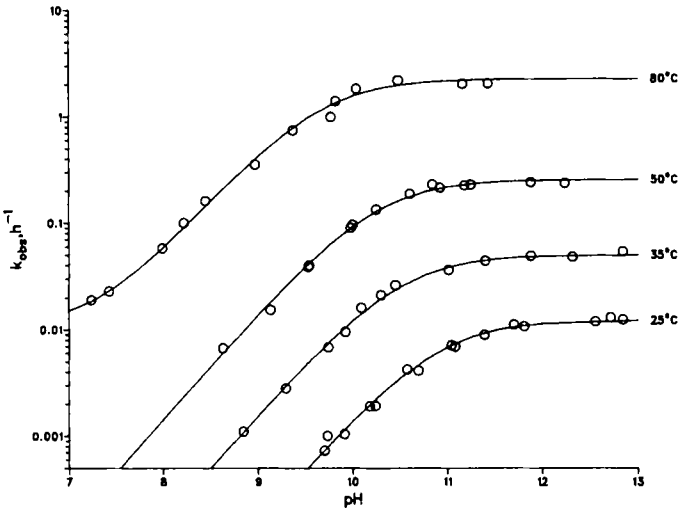


Figure 2—*Plots of k_{obs} versus pH for the hydrolysis of cibenzoline at the temperatures indicated. The lines were calculated using eq 1 and the data in Table II.*

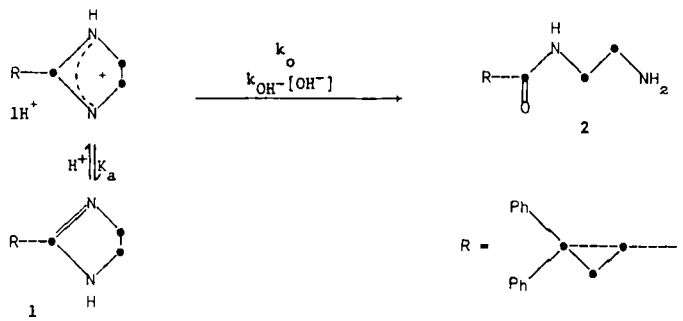
could only be detected at low pH and at the highest temperature. Although a mechanism involving the reaction of water with both the ionized and unionized forms of 1 is kinetically indistinguishable from the proposed mechanism, it is considered less likely on electrostatic grounds. The proposed mechanism is outlined in the Scheme and is described in eq 1.

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

where velocity = $v = k_o[1H^+] + k_{OH^-}[1H^+][OH^-]$.

The lines in Figure 2 are drawn using eq 1 and the kinetically determined pK_a values and rate constants shown in Table I. A plot of both the kinetic and potentiometric pK_a values versus temperature is shown in Figure 3. The gradient of the line is −0.021 and is similar to values obtained for other nitrogen heterocycles.[4]

The literature contains some disagreement regarding the mechanism of alkaline hydrolysis of simple imidazolines which are not substituted at both nitrogens. Martin and Parcell,[5] Saam and Baak,[6] and DeWolfe[7] favor the simple



Scheme

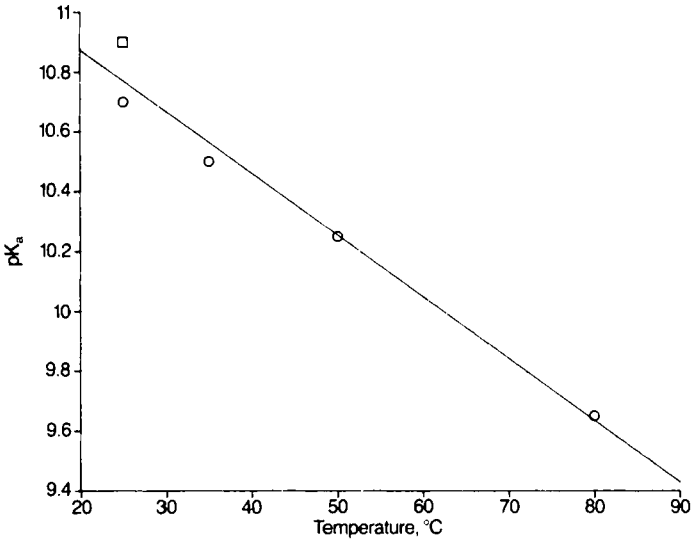


Figure 3—*Plot of pK_a values of the conjugate acid of cibenzoline versus temperature, obtained both kinetically (○) and potentiometrically (□).*

ionization mechanism outlined above. However, Harnsberger and Riebsomer,[8] in a study of the hydrolysis of a number of 1,2-disubstituted imidazolines, concluded that the mechanism involves the rapid and reversible reaction of free-base imidazoline with hydroxide ion to form an intermediate which reacts with water in the rate-determining step to give products. We are unable to fit our data for 1 to the rate equation corresponding to Harnsberger's mechanism. This is not unexpected, given the good fit of our data to eq 1, since our proposed mechanism and the Harnsberger mechanism differ in the dependence of observed rate constant on pH.

Fernandez et al.[9] studied the hydrolysis of 1-aryl-2-phenylimidazolines with different substituents in the aryl ring and concluded that their results supported the mechanism proposed by Harnsberger. The substituent effects observed by Fernandez and co-workers are actually in accord with a mechanism involving a rate-determining reaction between protonated imidazoline and hydroxide ion and do not support a mechanism in which free imidazoline reacts with either water or hydroxide ion. As discussed by Cordes and Jencks[10] in relation to the hydrolysis of Schiff bases, if the attack of water on the free base were rate-determining, the observed hydrolysis rates should be accelerated by electron-withdrawing substituents. Conversely, if the attack of hydroxide on the imidazolinium ion were rate-determining, the effects of substituents on the pre-equilibrium protonation would oppose those for the attack of hydroxide ion, and the observed hydrolysis rates would show little dependence on the nature of the substituents. However, if the reaction proceeded via the imidazolinium ion, electron-withdrawing substituents should increase the second-order rate constants k_{OH^-}. We find that the data of Fernandez and co-workers reveal little variation in the observed rate constants for hydrolysis, and that values of k_{OH^-}, calculated from Fernandez's data using eq 2 (eq 1 simplified to describe the situation at high pH),

$$k_{OH^-} = \frac{k_{obs} K_a}{K_w} \quad (2)$$

were found to increase with electron-withdrawing substituents (a 55-fold change between 4-OH and 4-NO_2). This suggests that the system studied by Fernandez and co-workers is best described by the mechanism we proposed for the hydrolysis of 1.

The hydrolysis of some *N,N*-disubstituted imidazolinium

ions has been shown to proceed via an observable tetrahedral "pseudobase" intermediate formed between the imidazolinium ion and hydroxide ion.[11] These reactions involve a general base-catalyzed rate-determining breakdown of the intermediate.[12] The HPTLC detection of a tetrahedral intermediate in the hydrolysis of 1-aryl-2-phenyl-imidazolines has also been claimed.[9] In an effort to look for such an intermediate in the cibenzoline case, we recorded ^{13}C NMR spectra during the course of a reaction in base. Cibenzoline succinate (100 mg) was dissolved in 2.2 mL of 1 M NaOH: CD_3OD (1:1.2), and ^{13}C NMR spectra were recorded at $t = 0$ and 24 h. The half-time for the reaction would be ~60 h at room temperature in the absence of any medium effects. Formation of a "pseudobase" in a rapid pre-equilibrium step would be associated with replacement of the imidazoline ring 2-C signal with a signal characteristic of a tetrahedral carbon. At $t = 0$ h, a signal for $>C = N$ at δ 168.4 ppm was observed, and, at $t = 24$ h, no additional signal was observed upfield. Thus, no evidence for the formation of a "pseudobase" intermediate in the case of 1 was found by NMR. This, and the lack of general base catalysis, suggest that either no intermediate exists, or formation of an intermediate is the rate-determining step in the hydrolysis of 1. Greater resonance in the *N,N*-diphenyl derivative may provide stability to the pseudobase which is not available in the unsubstituted species.

References and Notes

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Acknowledgments

The authors thank Mrs. J. Comment for typing the manuscript, Ms. A. Grant for the NMR work, and Mr. R. Dobies for help with drawing the figures.