Degradation of the Antiarthritic Prodrug, 3-Carboxy-5-methyl-*N*-[4-(trifluoromethoxy)phenyl]-4-isoxazolecarboxamide, in Aqueous Solution

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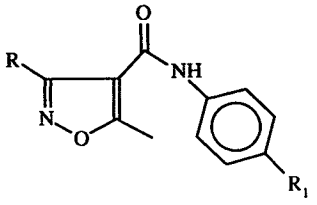
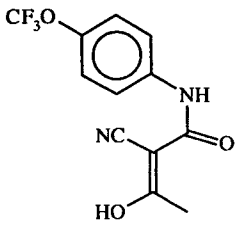
INTRODUCTION

3-Carboxy-5-methyl-*N*-[4-(trifluoromethoxy)phenyl]-4-isoxazolecarboxamide, **1**, is being examined as a potential prodrug for the antiarthritic agent 2-cyano-3-hydroxy-*N*-[4-(trifluoromethoxy)phenyl]-2-butenamide, **4**. It differs from the recently described compound 2-cyano-3-hydroxy-*N*-[4-(trifluoromethyl)phenyl]-2-butenamide, **2**, by the substitution on the phenyl ring (1). These compounds are metabolized to yield plasma concentrations of *N*-substituted 2-cyano-3-hydroxy-butenamides similar to the established prodrug, leflunomide, **3** (2). Stability studies of **1** in solution were initiated to support preliminary toxicology formulations. Also, by monitoring the concentration of **4** as a function of time, the kinetics and products of the active form have been elucidated so that these studies would provide insight into the transformation to the active form *in vivo*.



1. R = COOH, $R_1 = OCF_3$
2. R = COOH, $R_1 = CF_3$
3. R = H, $R_1 = CF_3$



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MATERIALS AND METHODS

Materials

Compound **1** was obtained from the Institute of Organic Chemistry, Syntex Research. *p*-Trifluoromethoxyaniline, **6**, was obtained from Fairfield Chemical Company. Distilled deionized water, ammonium acetate, potassium phosphate, potassium acetate (Mallinckrodt), potassium hydroxide solutions, and hydrochloric acid volumetric solutions (Aldrich) were used for preparation of the buffer solutions. All other chemicals used were of reagent grade.

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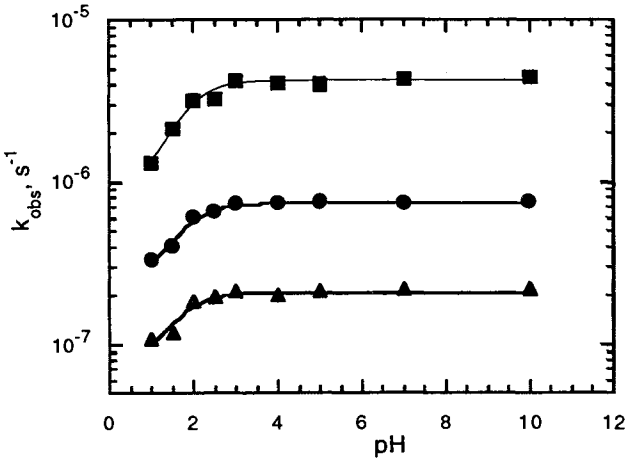
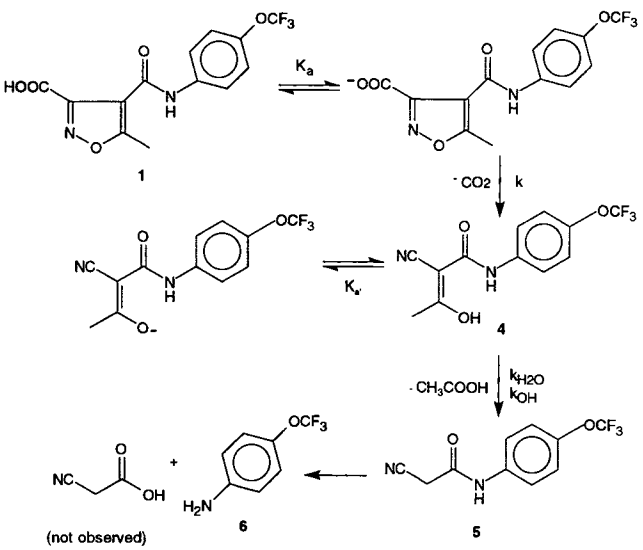


Fig. 1. pH–rate profile for the decomposition of **1** in aqueous solution at 40°C (▲), 50°C (●), and 60°C (■). The curves were drawn using Eq. (1) and the constants in Table I.

Degradation product **4** was prepared by reacting a 12.5 mg/mL solution of **1** at pH 10.0 and 100°C for 90 min. Upon cooling, a white precipitate formed, which was collected by filtration. Degradation product **5** was prepared by dissolving 25 mg of **1** in 2 mL of ethanol, 1 mL of water, and 0.1 mL of 1 *N* HCl. The sample was heated at 80°C for 72 hr. The ethanol was then removed by evaporation, which resulted in a precipitate. The precipitate was collected by filtration. Both structures and purity were confirmed by ^1H NMR, ^{13}C NMR, mass spectrometry, and HPLC.

Kinetic Methods

Aqueous solutions of **1** were prepared so that the final solution contained 38 μg/mL drug (~100 μ*M*). The buffers used were HCl (pH 1.0, 1.5, 2.0, 2.5, and 3.0), potassium



Scheme I

Table I. Calculated Rate Constants and Dissociation Constants for the Decomposition of 1 and 4 in Aqueous Solution

	1		4		
T (°C)	k ($10^4\ M^{-1}\ hr^{-1}$)	pK_a[a]	k_{H_2O} ($10^1\ hr^{-1}$)	k_{OH} ($10^{-5}\ M^{-1}\ hr^{-1}$)	pK_a'[a]
40	7	1.6	—[b]	4.3	2.7
50	28	1.7	1.1	9.0	2.9
60	148	1.8	3.0	15	2.7

[a] Determined by fitting kinetic data.
[b] A value could not be determined due to limited data.

acetate (pH 4.0 and 5.5), potassium phosphate (pH 7.0) and potassium carbonate (pH 10.0). With the exception of HCl, buffer concentrations were 0.01 *M*. Sample solutions were filtered through 0.22-μm Corning filters and flame-sealed in 2-mL clear ampoules. At selected time intervals, individual samples were removed from the elevated temperature ovens and stored at −18°C. All samples were assayed against a "zero-time" sample to determine the percentage drug remaining.

Curves were fit to the data using the general curve fitting function of KaleidaGraph (Synergy Software, Reading, PA) to determine the rate constants for the degradation of **4** and **5**. pH–rate profiles were also fitted with KaleidaGraph to determine apparent equilibrium constants and microscopic rate constants.

HPLC Methods

An HP 1050 (Hewlett Packard) HPLC system was used. The HPLC method used a Zorbax Rx (C8) column (5 μm, 4.6 × 250 mm), with a mobile phase of 0.1 *N* ammonium acetate (pH 6):acetonitrile (60:40). The detection wavelength, flow rate, and injection volume were 260 nm, 0.8 mL/min, and 20 μL, respectively. The retention times for **1**, **4**, **5**, and **6** were 7.0, 5.5, 18.5, and 20 min, respectively.

RESULTS AND DISCUSSION

Kinetics of Degradation of 1 in Aqueous Solution

The stability of **1** was studied in aqueous buffer solutions from pH 1 to pH 10 at 40, 50, and 60°C. The degrada-

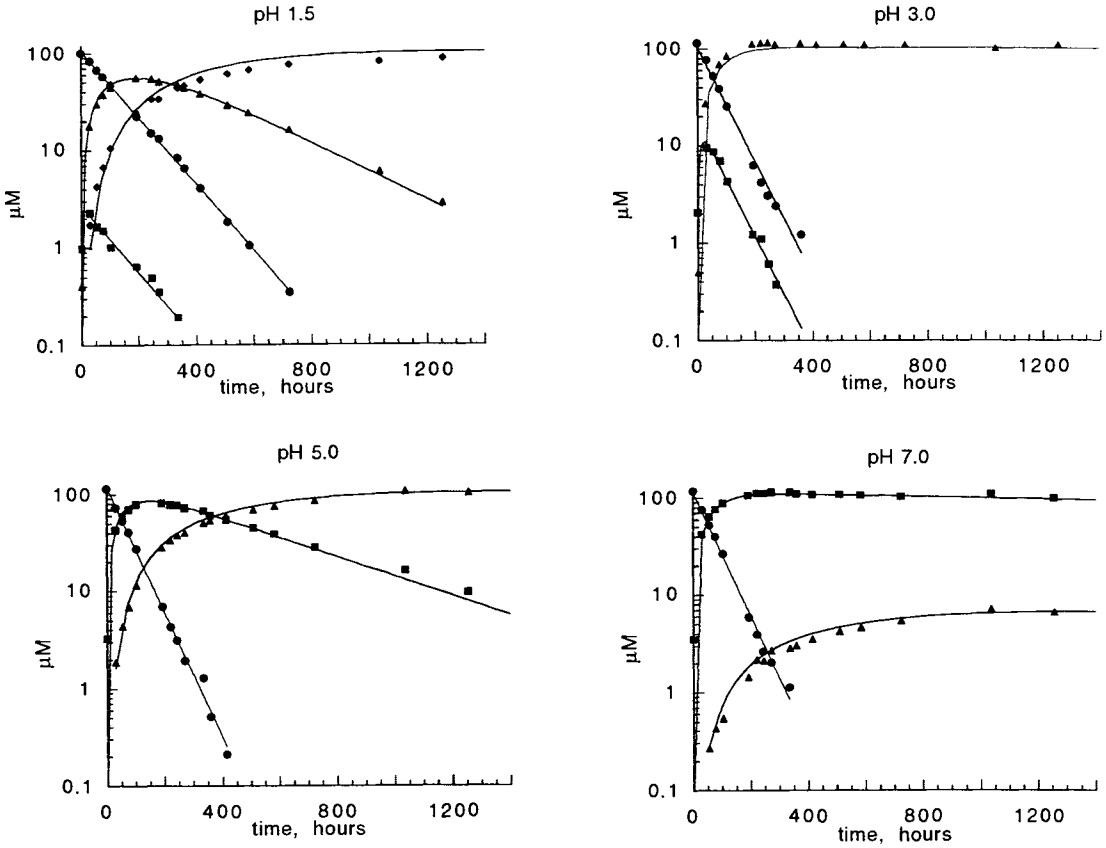


Fig. 2. Abundance of the components from the reaction of **1** in aqueous solution at 60°C: **1** (●), **4** (■), **5** (▲), and **6** (◆). The curves were drawn by fitting the data to Eqs. (2)–(4).

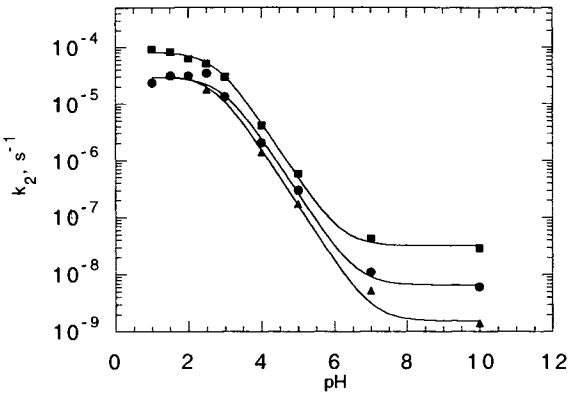


Fig. 3. pH–rate profile for the decomposition of 4 in aqueous solution at 40°C (▲), 50°C (●), and 60°C (■). The curves were drawn using Eq. (5) and the data in Table I.

tion kinetics were found to follow first-order kinetics for >2 half-lives. The pH dependence of the degradation rate at 40, 50, and 60°C are shown in Fig. 1.

The pH–rate profile can be explained by a reaction mechanism (see Scheme I) involving the decomposition of the conjugate base of 1 (3–6). The observed rate constant, k_{obs}, can be expressed in terms of the microscopic rate for the decomposition of the conjugate base of 1, k, and the dissociation constant of 1, K_a, as shown in Eq. (1).

$$k_{obs} = \frac{kK_a}{K_a + [H^+]} \tag{1}$$

Values for the apparent microscopic rate constants and the dissociation constant at different temperatures are summarized in Table I. The pK_a values obtained from the kinetic data are comparable to the pK_a value of 1.9 determined from solubility measurements at 25°C. The solid curves drawn in Fig. 1 were constructed from these apparent rate and dissociation constants. When the data are extrapolated to 25°C a t_{90} of approximately 3 months is predicted from pH 3 to pH 10. The reactivity of 1 in the pH region studied ($t_{1/2}$ = 70 days at 37°C) cannot explain the *in vivo* conversion to the active form (1), suggesting that the reaction is mediated by biological catalysts (6).

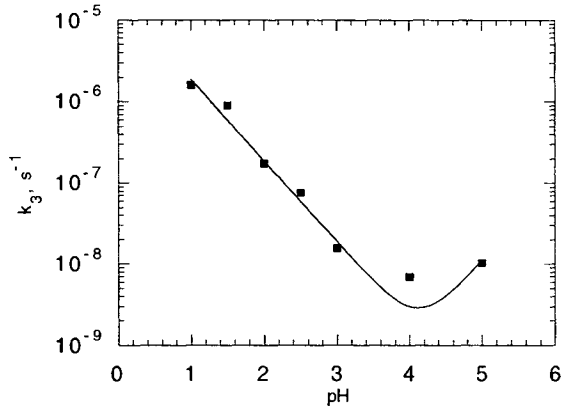


Fig. 4. pH–rate profile for the decomposition of 5 in aqueous solution at 60°C.

Degradation Products

The degradation products from the decomposition of 1 in aqueous solution were identified by HPLC by coinjecting authentic samples (see Materials and Methods). In the pH range examined, HPLC analysis detected the presence of three products: the active form of the drug, 4; *N*-(*p*-trifluoromethoxy)phenyl-2-cyano-acetamide, 5; and *p*-trifluoromethoxyaniline, 6. The relative concentrations of the three components as a function of pH, reaction time, and temperature are shown in Fig. 2.

Kinetics of Degradation of 4 and 5

To determine the rate constants for the disappearance of 4 and 5, the concentrations of the observed products as a function of time can be analyzed as the following kinetic scheme:

$$1 \xrightarrow{k_{obs}} 4 \xrightarrow{k_2} 5 \xrightarrow{k_3} 6$$

The concentrations of 4 [4], 5 [5], and 6 [6] can be described by the following integrated equations (7):

$$[4] = \left\{\frac{k_{obs}[1]_{t=0}}{k_2 - k_{obs}}\right\}\{e^{-k_{obs}t} - e^{-k_2 t}\} \tag{2}$$

$$[5] = [1]_{t=0}\left\{\frac{k_2 k_{obs} e^{-k_{obs}t}}{(k_2 - k_{obs})(k_3 - k_{obs})} + \frac{k_{obs}k_2 e^{-k_2 t}}{(k_{obs} - k_2)(k_3 - k_2)} + \frac{k_{obs}k_2 e^{-k_3 t}}{(k_{obs} - k_3)(k_2 - k_3)}\right\} \tag{3}$$

$$[6] = [1]_{t=0}\left\{1 - \frac{k_2 k_3 e^{-k_{obs}t}}{(k_2 - k_{obs})(k_3 - k_{obs})} - \frac{k_{obs}k_3 e^{-k_2 t}}{(k_{obs} - k_2)(k_3 - k_2)} - \frac{k_{obs}k_2 e^{-k_3 t}}{(k_{obs} - k_3)(k_2 - k_3)}\right\} \tag{4}$$

To obtain k_2 or k_3, it is necessary only to best fit at least one of the appropriate integrated equations to the concentration versus time data for the degradation products since values for k_{obs} were determined previously.

The pH dependence of the decomposition of 4, k_2, at 40, 50, and 60°C obtained by curve fitting the data is shown in Fig. 3. The pH–rate profile can be fitted by an equation that has terms arising from water and hydroxide catalyzed decomposition of the neutral form of 4 (8,9). This equation can alternatively be interpreted as having terms due to the water-catalyzed decomposition of the neutral and ionized species.

Equation (5) expresses the macroscopic rate constant, k_2, in terms of the microscopic rate and equilibrium constant, k_{H_2O}, k_{OH}, and $K_{a'}$, and the hydrogen ion concentration (8).

$$k_2 = \frac{k_{H_2O}[H^+]}{[H^+] + K_{a'}} + \frac{k_{OH}K_w}{[H^+] + K_{a'}} \tag{5}$$

Table I gives the values obtained for k_{H_2O}, k_{OH}, and p$K_{a'}$ obtained by fitting Eq. (5) to the data. The data predict a t_{90} of approximately 20 and 5 hr at 25 and 37°C, respectively, in

the region below pH 3. The predicted pK_a value of 2.8 is comparable to the pK_a value of 3.4 for phenyl-2-cyanoacetamide (10). The low pK_a of the active form of the drug may have biological implications, since at pH values above 2.8, the molecule would be in the ionized form.

The rate of formation of 6 from 5, k_3, can also be determined in the pH region where this reaction can be observed. The pH profile (Fig. 4) shows acid catalysis terms as expected for anilide hydrolysis (11).

ACKNOWLEDGMENTS

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