Kinetics and Mechanism of Degradation of Zileuton, a Potent 5-Lipoxygenase Inhibitor

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Received April 6, 1992; accepted April 27, 1992

Zileuton (*N*-(1-benzo[*b*]thien-2-ylethyl)*N*-hydroxyurea) is a powerful 5-lipoxygenase inhibitor. The chemical degradation of Zileuton and related hydroxyurea derivatives was studied in aqueous solutions as a function of pH and temperature. The pH profile for the degradation of Zileuton shows an acid-catalyzed region at pH values below 2, water hydrolysis of the protonated form at pH values from 3 to 8, and water hydrolysis of the unprotonated form at pH values greater than 9. Hydrolysis of the hydroxyurea moiety to give the hydroxylamine derivative represents the main degradation pathway for Zileuton. This product, however, is not stable and is present at low concentrations at pH values below 6 and not observed at pH values greater than 7. Further decomposition of the hydroxylamine derivative leads to the observed degradation products. Air oxidation to the isomeric oximes accounts for the observed products at pH values greater than 7. Hydrolysis of the oximes to the ketone derivative accounts for the observed products at pH values 2 to 6. Parallel decomposition pathways to the alcohol derivative were noted under strongly acidic conditions, pH 0 to 2.

KEY WORDS: lipoxygenase; 5-lipoxygenase inhibitor; kinetics; borate catalysis; *N*-hydroxyurea.

INTRODUCTION

Arachidonic acid, 5,8,11,14-eicosatetraenoic acid, is the precursor of a large number of important immunoregulators such as prostaglandins and leukotrienes (1–3). Since leukotrienes are involved in defense mechanisms such as inflammation and hypersensitivity responses, it is thought that control of their biosynthesis can lead to successful treatment of a variety of allergic ailments such as asthma, psoriasis, arthritis, Krone's disease, etc. The first step in the metabolism of arachadonic acid to leukotrienes involves oxidation of the polyunsaturated acid by a 5-lipoxygenase (5-LO) enzyme, to yield the hydroperoxide derivative 5-hydroxyperoxytetraenoic acid, 5-HPETE, which in turn is dehydrated to the epoxide leukotriene A_4 (LTA_4). Further metabolism of LTA_4 leads to the formation of other leokotrienes such as LTB_4, LTC_4, LTD_4, and LTE_4, all found to possess proinflammatory activity (1,2). Reports in the literature show that several types of hydroxamic acids derivatives are powerful inhibitors of 5-LO in both *in vitro* and *in vivo* experiments (4–7). Similar hydroxyurea derivatives (8) are particularly potent inhibitors of 5-LO. The title compound, zileuton, is a powerful lipoxygenase inhibitor belonging to the hydroxyurea family (9). The kinetics and mechanism of degradation of Zileuton in aqueous solutions were investigated and are described in this paper.

MATERIALS AND METHODS

Chemicals

The compounds (Scheme I) *N*-(1-benzo[*b*]thien-2-ylethy)-*N*-hydroxyurea (I, or Zileuton), *N*-(1-benzo[*b*]thien-2-ylethy)-hydroxylamine (II); 1-benzo[*b*]thien-2-ylethanol (III), 1-benzo[*b*]thien-2-ylethanone oxime (IV), and 1-benzo[*b*]thien-2-ylethanone (V) were obtained from Analytical Research Services at Abbott. Elemental and spectral analysis of I are consistent with the structure shown in Scheme 1. The purity of Zileuton used in these experiments [Lots 99-078-AX (anhydrous) and 10-753-AL (monohydrate)] is higher than 99% as judged by HPLC and differential scanning calorimetry (DSC). The derivatives of Zileuton, compounds II to V, were used primarily for identification purposes. With the exception of IV, all of them showed a single peak under the HPLC conditions employed. Compound IV was received as a mixture of syn and anti oximes (syn-IV and anti-IV) at an approximately 1:1 molar ratio. All chemicals employed in the preparation of buffers were analytical reagent grade. Buffers were prepared in deionized-distilled water (Milli-Q water system from Millipore). The following buffers were employed: HCl/KCl, pH 0 to 2; citrate, pH 3 to 6; phosphate, pH 7 to 8; carbonate or borate, pH 9 to 11; and NaOH/NaCl, pH values greater than 12. The total buffer concentration was 0.050 *M*, and the ionic strength, *I*, was kept at 0.2 *M* (except citrate buffer, pH 6, where $I = 0.26\ M$) with KCl or NaCl. Although the reported pH values were determined at room temperature, no significant correlations were found necessary at the higher temperatures of the kinetic studies.

Analytical Methods

The concentration of Zileuton and its degradation products was determined by HPLC. The analytical columns employed during the course of these experiments were (a) an Adsorbosphere C-18 (Alltech Associates, 15 cm, 4.6-mm OD, 3-μm particles), (b) an Adsorbosphere-HS C-18 (Alltech Associates, 25 cm, 4.6-mm D, 5-μm particles), or (c) a Nova-Pak C-18 column (Waters, 15 cm, 4.6-mm OD, 3-μm particles). The mobile phase consisted of methanol:water:triethylamine at a 60:40:1 ratio brought to pH 7 with phosphoric acid (columns a and b) or at a 54:46:1 ratio (column c). The flow rate was 0.75 or 0.80 ml/min. The use of different analytical columns led to changes in the order of elution of the peaks. With column a, V elutes between anti-IV and syn-IV, while with column b or c, V elutes before IV. The differences in the order of elution and the changes in retention times of the peaks proved useful in characterizing the degradation peaks when their retention times were compared with those from authentic samples. Other HPLC instrumentation involved a Spectra-Physics pump (SP-8770 or SP-8700), a Waters autoinjector, WISP 710B, a UV detector (Kratos Spectroflow 783) set at 260 nm, and a recording integrator (Spectra Physics SP-4270).

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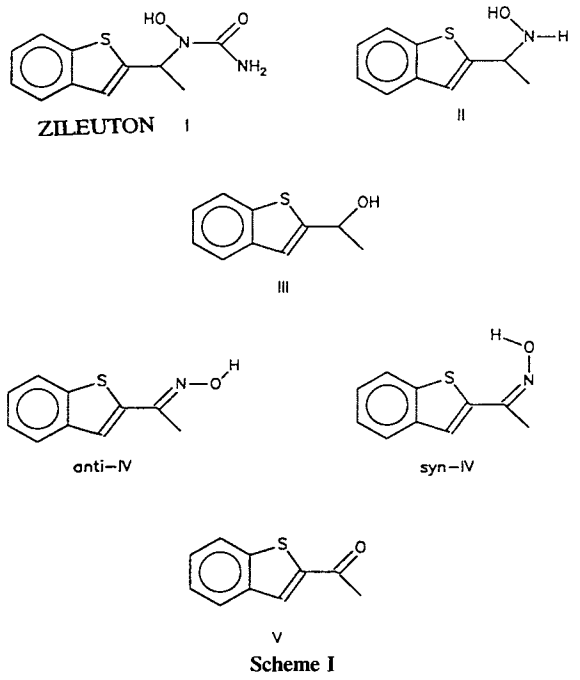


Scheme I

pKa Determination

Fifty microliters of a stock solution of I in methanol (4.23 m*M*) was mixed with 2450 μl of buffer and the absorbance at 260, 270, and 280 nm was recorded with a Hewlet Packard Model 8451-A spectrophotometer. The pK_a was determined by nonlinear least-squares fit of the data to a model for the dissociation of a monoprotic acid as a function of pH. Similar values were obtained at the three wavelengths.

Kinetics

Hydrolysis Rate Studies

Stock solutions of I in methanol were diluted 50- to 100-fold with buffers (0.050 *M;* ionic strength, 0.2 *M*) to give final concentrations of about 0.05 to 0.1 m*M*. Aliquots of these solutions were transferred to glass ampoules and promptly sealed. The ampoules were placed in constant-temperature ovens (Blue M, Blue Island, IL) kept at 51, 61, and 82°C (all ±1°C). One or two ampoules were withdrawn at selected times and placed in a refrigerator. Control experiments showed that nonmeasurable degradation took place while the samples were stored at 4°C. All the samples were analyzed by HPLC at the same time. Before analysis, the solution from the ampoule was diluted three to five times with mobile phase that had been brought to pH 4 with HCl. It was found necessary to lower the pH of the diluting solvent to quench the oxidation of II during analysis. Concentrations of Zileuton and other degradation products were calculated from standard curves. Correlation coefficients for the standard curves were better than 0.999 and showed no significant intercept. The rate constants were calculated from the slopes of the plots of logarithm of concentration (or peak area using the HPLC integrator) versus time. These plots were linear for over five half-lives. The effect of buffer concentration on the rate of hydrolysis of Zileuton was investigated at pH 3 (citrate), 7 (phosphate), and 10 (carbonate, borate) by varying the total buffer concentration between 0.050 and 0.50 *M*. The ionic strength of the solutions was kept constant at 1.0 *M* with KCl. The reactions were carried out at 61 or 82°C as indicated above.

Oxidation of Hydroxylamine Derivative II to Syn-IV and Anti-IV

The degradation of II was studied from pH 7 to 12 at 25°C and from pH 1 to pH 5 at 82°C. The procedure employed for the reactions at 82°C was essentially the same as described earlier for the hydrolysis of I. The following procedure was employed for the reactions at 25°C. A stock solution of II in MeOH (5.71 m*M*) was diluted 50-fold with buffer in an Erlenmayer flask. Aliquots (1.0-ml) were withdrawn at selected times and mixed with 2.0 ml of mobile phase and 0.1 ml of 1.0 *N* HCl (alternatively, the 1.0-ml aliquot was mixed with 2.0 ml of mobile phase that had been previously acidified to pH 4). The samples were analyzed by HPLC using the same system described earlier.

The effect of ambient-air oxygen on the rate of oxidation of II to syn-IV and anti-IV was studied according to the following procedure. A borate buffer, pH 9, was deoxygenated by bringing it to a boil and carefully cooling it down to room temperature while keeping a constant flow of high-purity nitrogen (>99.99%) through the solution. Twenty-five-milliliter aliquots of the buffer were mixed with 0.50 ml of a stock solution of II (previously degassed with helium) and purged with either high-purity nitrogen or oxygen. One-milliliter aliquots were taken, mixed with 2.0 ml of mobile phase and 0.1 ml of 1.0 *N* HCl, and immediately analyzed by HPLC.

Hydrolysis of Syn-IV and Anti-IV to V

The hydrolysis of a mixture of syn- and anti-IV at an approximately 1:1 molar ratio, to the corresponding ketone V was studied in the acidic pH range, 1 to 6, at 82°C. Solutions of IV, overall 0.27 m*M*, were sealed into ampoules and placed in an 82°C oven following the procedure described earlier. The HPLC assay of the solutions at the end of the reaction was the same as that discussed previously for the other compounds.

RESULTS AND DISCUSSION

It was suspected that decomposition of Zileuton in aqueous solution should proceed primarily through hydrolysis at the *N*-hydroxyurea moiety. In general, the hydrolysis of *N*-hydroxyurea compounds such as I is expected to result in the formation of the single product II. Although decomposition of the tetrahedral intermediate that results from water addition to the carbonyl group might also lead to the formation of the *N*-carboxy acid, this compound is not expected to be stable in the reaction medium and decarboxylation of this reactive intermediate should be fast. For example, Heyns *et al.* (10) have shown that decarboxylation of the *N*-carboxyl intermediates formed during the polymerization of 2,5-oxazolidinediones does not show a carbon-13 isotope effect, although isotopic fractionation in the decarboxylation step is expected. Since several products are observed during

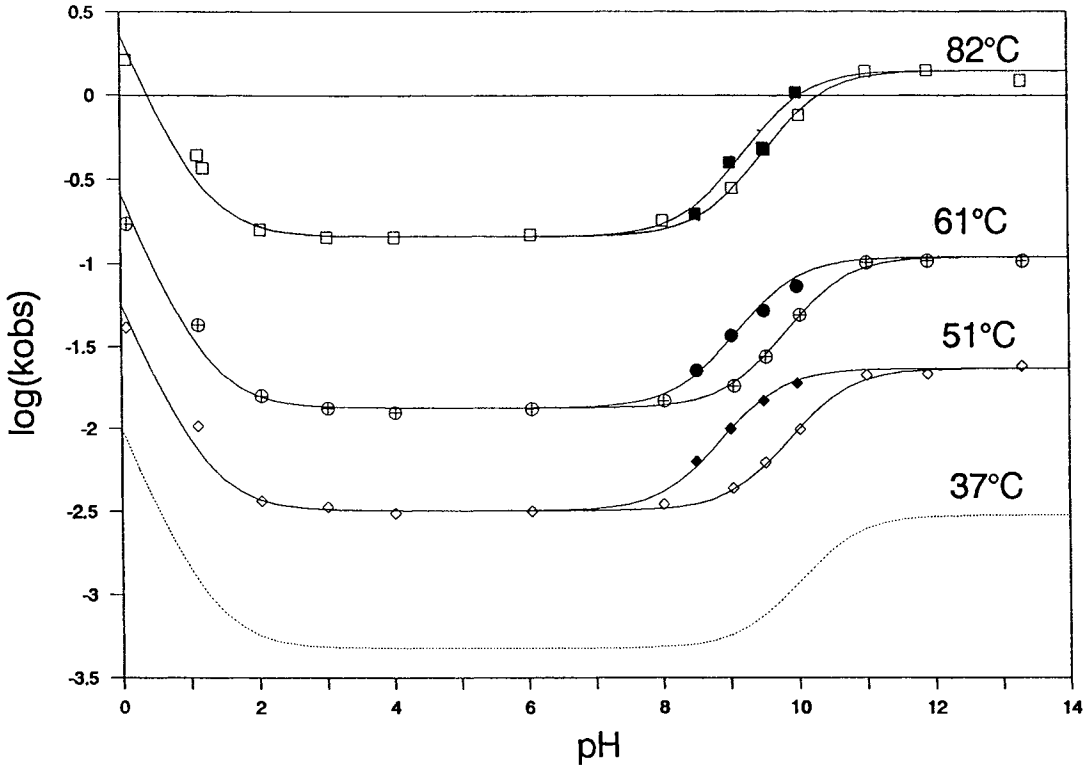


Fig. 1. pH profile for the hydrolysis of Zileuton (~0.2 m*M*) at different temperatures. The filled symbols represent the observed rates in the presence of borate. The solid lines are theoretical lines calculated using the parameters from a fit of the pH–rate data to Eq. (1). The dotted line is an extrapolated line at 37°C using the activation parameters in Table II. The following buffers (0.05 *M*) were used: HCl/KCl, pH 0–2; citrate, pH 3 to 6; phosphate, pH 7–8; carbonate or borate, pH 8.5 to 11; and NaOH/KCl, pH >12. Ionic strength, 0.2 *M*.

Table I. Calculated Acid- and Water-Catalyzed Rate Constants for the Hydrolysis of Zileuton at Different Temperatures and the Corresponding Activation Parameters[a]

T (°C)	k_{H^+}, M^{-1} hr^{-1} (SD)	$k_{1(HOH)}$, hr^{-1} (SD)	$k_{2(HOH)}$, hr^{-1} (SD)	pK_a (SD)
82	2.20 (0.20)	0.15 (0.01)	1.44 (0.06)	10.05[b] (0.08)
61	0.25 (0.02)	0.013 (0.001)	0.111 (0.005)	10.28[b] (0.07)
51	0.055 (0.003)	0.0032 (0.0003)	0.023 (0.001)	10.31[b] (0.09)
37	9.35E-03	4.75E-04	2.99E-03	
25	1.63E-03	7.66E-05	4.18E-04	10.51[c] (0.05)
82				9.72[d] (0.11)
61				9.56[d] (0.09)
51				9.32[d] (0.10)

	Activation parameters		
	k_{H^+}	$k_{1(HOH)}$	$k_{2(HOH)}$
ΔH (kcal/mol)	26.1 (2.1)	27.3 (1.0)	29.5 (1.2)
ΔS (eu)	0.2 (6.2)	−2.0 (3.0)	8.8 (3.6)
ΔG (kcal/mol)	26.2	26.7	32.1

[a] Standard deviations for the activation parameters are indicated in parentheses. Free energies of activation were calculated at 25°C.
[b] Apparent pK_a for Zileuton obtained from a fit of the kinetic data to Eq. (1).
[c] pK_a was determined spectrophotometrically at 25°C.
[d] Apparent pK_a for Zileuton in the presence of 0.05 *M* borate.

Table II[a]

	T (°C)	pH	k_{water},[b] hr^{-1} (SD)	k_{buffer},[b] M^{-1} hr^{-1} (SD)
Water	61	7.0	0.012 (0.001)	
$k_{1(HOH)}$[c]	61	3–8	0.013 (0.001)	
Carbonate	61	10.03	0.052 (0.004)	0.06 (0.02)
Borate	61	10.02	0.35[d] (0.05)	0.32[e] (0.03)
Citrate	82	3.07	0.147 (0.004)	0.35 (0.01)
Phosphate	82	7.04	0.158 (0.011)	0.39 (0.04)
Carbonate	82	10.03	0.86 (0.03)	0.38 (0.09)

[a] [Zileuton] = 0.17 mM. Buffer concentration was caried from 0.05 to 0.5 M. I = 1.0 M.
[b] Intercept and slope of the linear regression fit of the observed rate constant as a function of buffer concentration.
[c] k_{water} at 0.05 M buffer from a fit of the pH–rate data to Eq. (1).
[d] k_{bor}, Dissociation constant (M) for Zileuton borate complex according to Eq. (2).
[e] k_{bor}, Maximal hydrolysis rate of Zileuton borate complex (hr^{-1}) under saturating borate concentration.

degradation of I at different pH values, it was important to find out if more than one mechanistic pathway is responsible for the degradation of this compound in solution.

pH–Rate Profile and Temperature Dependence

The pH–rate profile for the hydrolysis of Zileuton is shown in Fig. 1. In the pH range 2 to 8 the observed first-order rate constant is independent of pH. At pH values below 2 the rate increases proportionally with the hydrogen ion concentration in an apparent specific acid-catalyzed reaction. Above pH 8 the rate increases with hydroxide concentration and levels off at higher pH values, describing a typical titration curve. This change in rate with pH coincides with the pK_a of the OH group in the N-hydroxyurea moiety. The pK_a was determined independently by a spectrophotometric technique (pK_a = 10.50 ± 0.05, 25°C) and agreed well with the extrapolated pK_a at 25°C using the calculated parameters from the pH–rate data (Table I). The kinetic data as a function of pH were fitted to Eq. (1) to estimate the acid- and water-catalyzed rate constants.

$$k_{obs} = k_{H^+}[H^+] + k_1 f_{(AH)} + k_2 f_{(A^-)} \quad (1)$$

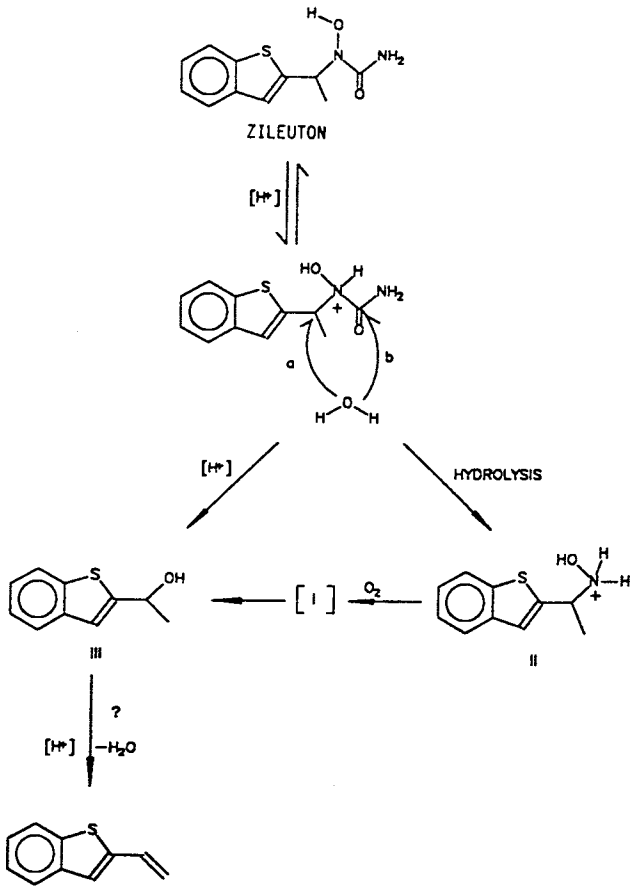
The constant k_{H^+} is the specific acid-catalyzed rate constant, $f_{(AH)}$ and $f_{(A^-)}$ are the fractions of Zileuton in the protonated and monoanion forms respectively, and k_1 and k_2 are the neutral or water-catalyzed hydrolysis rate constant for each of the above forms. The fraction of protonated form is given by $f_{(AH)} = [H^+]/([H^+] + K_a)$ and $f_{(A^-)} = 1 - f_{(AH)}$. The parameters obtained from a fit of the data to Eq. (1) are collected in Table I. Eyring plots of the calculated parameters are linear in the temperature range studied (not shown) and were used to estimate the rate constants for the hydrolysis of Zileuton at room temperature. Activation energies and enthalpies were similar for the acid-catalyzed and water reactions and are about 27 kcal/mol. A similarly high activation enthalpy was observed for the water-catalyzed hydrolysis of urea and some of its derivatives (11,12).

The observed rate constants were not extrapolated to zero buffer concentration. The effect of buffer concentration on the hydrolysis rate was examined using different buffer species and pH values. The buffer dependencies at pH 3, 7,

Table III. Response Factors for Zileuton and the Observed Degradation Products[a]

Compound	MW	RF (SD)
Zileuton	236	1.00 —
II	193	0.80 (0.05)
IV	191	0.84 (0.07)
V	176	0.38 (0.04)
III	178	0.81 (0.01)

[a] The response factors (RF) are normalized with respect to Zileuton. They represent the relative sensitivity, on a molar basis, for each compound at the wavelength (260 nm) of the HPLC assay.



Scheme II

and 10 at 82°C (ionic strength, 1.0 *M*) are linear and the extrapolated rate constants at zero buffer concentration are less than 10% smaller than the observed rate constant at the buffer concentration employed during the kinetic runs (Table II). This was verified independently by measuring the rate of hydrolysis of Zileuton in water, pH ~7, in the absence of any buffer species (Table II).

Electrophilic Catalysis of Zileuton Hydrolysis by Borate Ion

The tendency of borate ions to form complexes with alcohols, particularly *cis* 1,2- or 1,3-diols (13,14), could affect the rate of Zileuton hydrolysis by complexation to the oxygens of the hydroxyurea moiety. Hydrolysis rates at a constant pH were greater in the presence of borate than carbonate ion. The rate increase with carbonate concentration is approximately linear, while the same effect with borate apppears to follow saturation behavior (Table II). This is thought to result from the complexation of the borate ion to the hydroxy group in the hydroxyurea, rendering a more electrophilic, and therefore more reactive, carbonyl group. Neglecting aggregation or polymerization of borate ions as a function of concentration (15), a simple equilibrium binding model can be used to deduce the effect of borate concentration on the rate of hydrolysis of Zileuton [Eq. (2)].

$$k_{obs} = k_o + \frac{k_{bor} * [\text{borate}]}{K_{bor} + [\text{borate}]} \quad (2)$$

The value for k_o, the observed rate constant in the absence of borate ion, can be extrapolated from the observed buffer dependence with carbonate ion at zero buffer concentration. The rate constant k_{bor} is the maximal rate under saturating borate concentrations and K_{bor} is the dissociation constant for borate complexation to Zileuton. K_{bor} was estimated at about 0.3 *M* from the nonlinear regression fit of the data to Eq. (2).

The effect of borate was also studied at different pH values and temperatures and is summarized in Table I and Fig. 1. These pH profiles were fitted using Eq. (1). The apparent effect of borate is to shift the pK_a of Zileuton by about one-half pK_a unit to more acidic values. These calculated pK_a values are summarized in Table I.

Product Distribution

A detailed analysis of the product distribution as a function of pH, temperature, and fraction of reaction was carried out to help elucidate the mechanisms that lead to the observed degradation products. It was noted that response factors obtained from standards of each of the observed products (Table III) could be used successfully to account for over 95% of the initial concentration of Zileuton. An exception was noted in strongly acidic solutions (pH ~0), where a very late-eluting peak was observed in the chromatograms. Due to its very nonpolar properties it was suggested that this compound is the dehydration product formed from the alcohol I (Scheme II). Figure 2 shows the product distribution as a function of pH, at 61°C, when approximately 90% of Zileuton has been consumed. The observed product distribu-

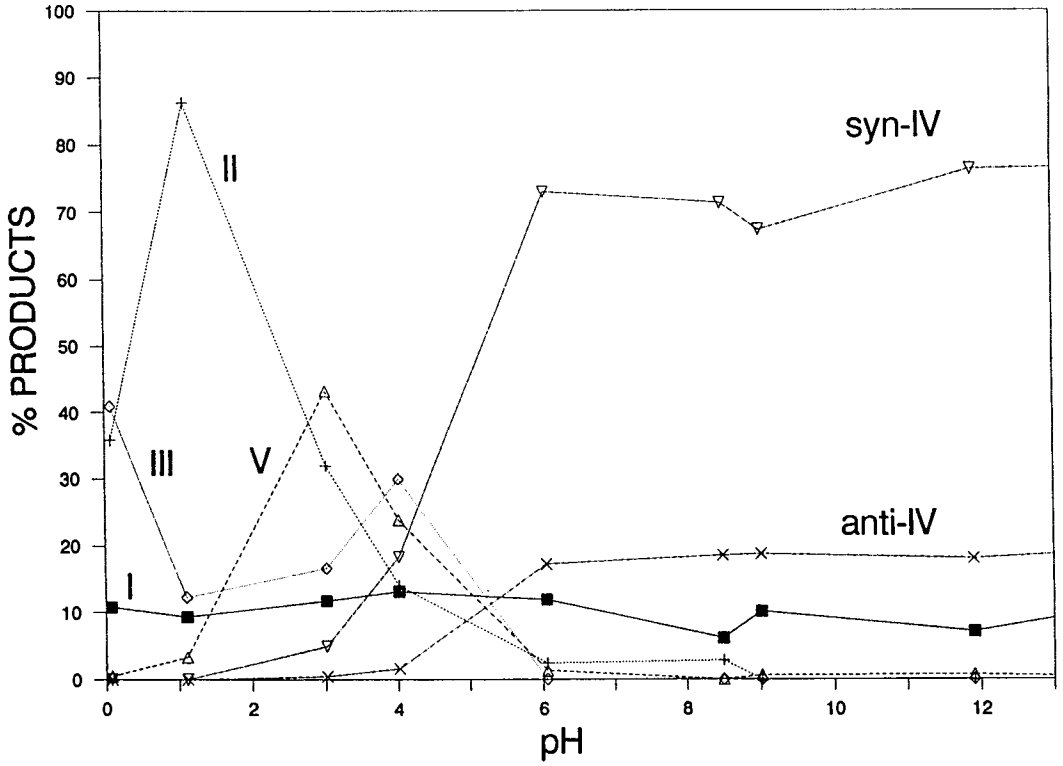


Fig. 2. Calculated product distribution as a function of pH at 61°C. The molar percentage of each of the observed degradation products when approximately 10% of Zileuton remains in the reaction mixture is shown, calculated using the response factors indicated in Table IV. Similar pH dependencies for products distribution were observed at other temperatures.

tion indicates that at pH 0 and 1 the alcohol derivative (III) and the hydroxylamine (II) are the major products. Between pH 2 and pH 6 several products are observed in different proportions. However, the following trends are observed. The concentration of oximes increases with pH, while those of II and III decrease. The concentration of ketone V appears to be maximal around pH 3 to 4. At pH values greater than 6, the syn and anti oximes are the only observed products. The time dependence for product formation was studied at each individual pH in an effort to unravel this complex product distribution (Fig. 3).

Under strongly acidic solutions, pH 0 and 1, Zileuton appears to degrade by parallel pathways that lead directly to hydroxylamine II and alcohol III. For example, at pH 1 and 61°C, the rate of formation of II and III (0.042 and 0.043 hr^{-1}, respectively), is approximately the same as the rate of disappearance of Zileuton (0.043 hr^{-1}). At higher pH values, III appears to form predominantly through the intermediate hydroxylamine II. This is supported by the fact that degradation of II also yields III and that the overall yield of III reaches a maximum at approximately pH 4 (Fig. 2). Formation of III is expected to result from nucleophilic substitution at the secondary carbon. In this case, departure of the leaving group, the *N*-hydroxyurea, is favored by protonation of the amide nitrogen in the reactant, whose pK_a values are estimated to be about −4 to 0 (16). This probably explains why the degradation of Zileoton to form III is not significant until the pH is close to 0. Formation of the alcohol from the hydroxylamine is believed to follow a different mechanism since the proportion of alcohol formed increases with pH, contrary to what is expected for a reaction that is facilitated by protonation. Also, the rate of degradation of II is pH independent from 1 to 5 (k_{obs} = 0.08 ± 0.01 hr^{-1} at 82°C; Table IV), while formation of the observed degradation products is markedly pH dependent (Fig. 2).

The two isomeric oximes are the only products observed after degradation of Zileuton or II at pH values greater than 7. Since the rate of oxidation of the hydroxylamine to oximes is much faster than the rate of hydrolysis of Zileuton, little hydroxylamine accumulates under the reaction conditions. The rate of oxidation of II increases with pH to a maximum at about pH 9, where its half-life at 25°C is only 10 min, five orders of magnitude faster than the rate of hydrolysis of Zileuton under these conditions (the calculated half-life for Zileuton hydrolysis at pH 9 is approximately 10 months). However, if the basic hydrolysis of Zileuton is rapidly quenched with strong acid, a small amount of hydroxylamine can be trapped. This is more noticeable in the pres-

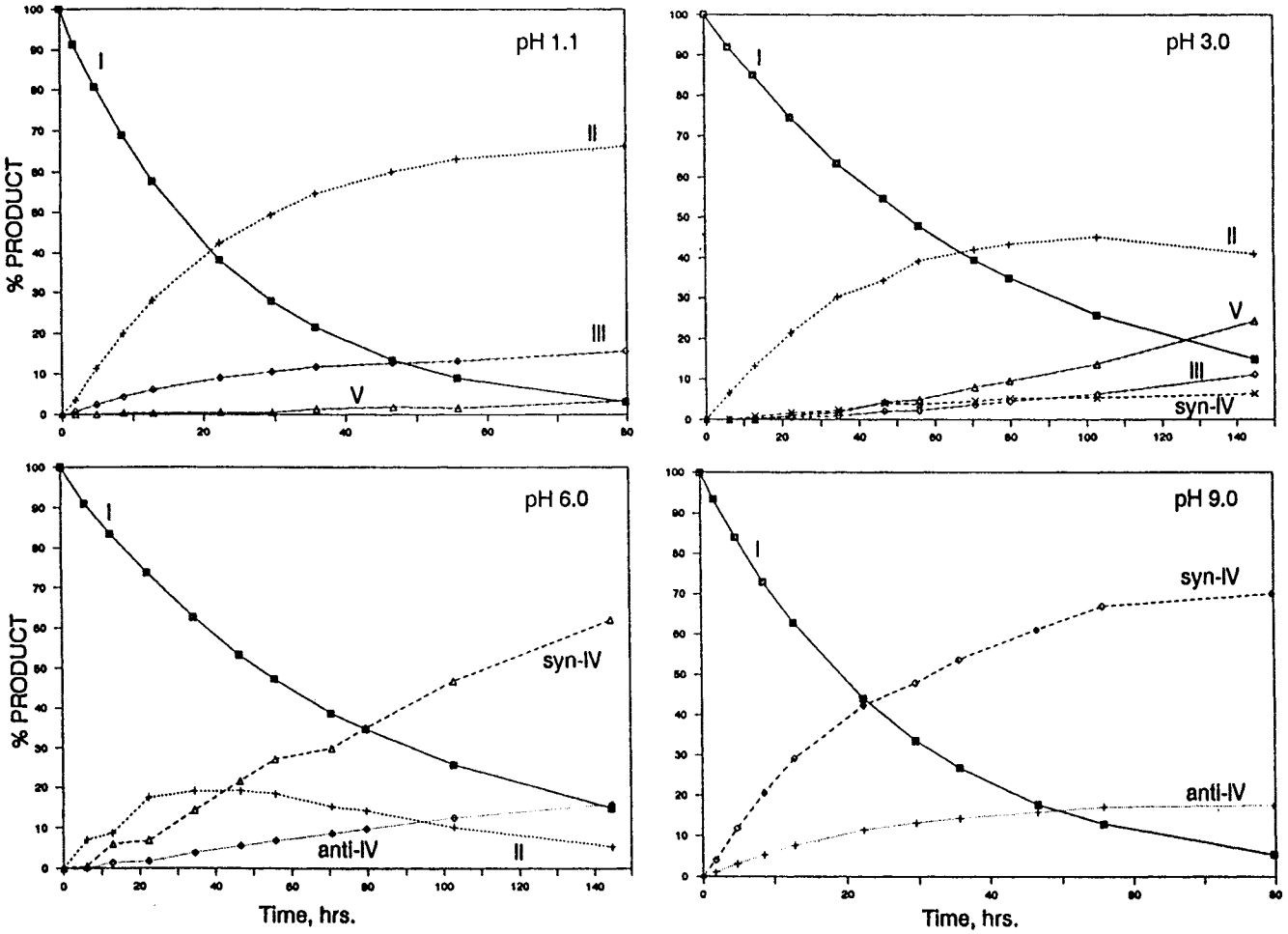


Fig. 3. Time dependence for the disappearance of Zileuton and the appearance of its degradation products as a function of pH at 61°C. Similar profiles were observed at other temperatures.

Table IV. Air Oxidation of Zileuton Hydroxylamine (II) to the Syn and Anti Oximes as a Function of pH[a]

pH	Temp. (°C)	k_{obs}, hr^{-1} (SD)	$t_{1/2}$, hr	syn/anti ratio
1.1	82	0.068 (0.003)	10.2	—[b]
3.0	82	0.092 (0.005)	7.5	>20[c]
5.0	82	0.092 (0.002)	7.5	>7[c]
		k_{obs}, min^{-1} (SD)	$t_{1/2}$, min	
7.0	25	0.0052 (0.0001)	134	3.95
8.0	25	0.0120 (0.0002)	58	3.97
9.0	25	0.0761 (0.0027)	9	3.64
10.0	25	0.0070 (0.0002)	100	3.66
12.0	25	0.0057 (0.0001)	121	4.02

[a] [II] = 0.2 m*M*; [buffers] = 0.05 *M*; *I* = 0.2 *M*.
[b] No anti oxime was observed.
[c] The syn/anti ratio increases with the extent of reaction. The reported values were observed when more than 90% of II had been consumed.

ence of borate buffers, suggesting that borate might be able to complex II and help to stabilize this compound to further oxidation (data not shown). The ratio of syn to anti oximes formed at pH values greater than 6 is always constant and approximately equal to four in favor of the syn isomer (Table IV). The increased stability of the syn isomer probably results from intramolecular hydrogen bonding between the OH hydrogen of the syn oxime and the sulfur atom in the ring. The ratio of syn/anti oximes changes slightly with temperature following a normal temperature dependence, for example, the observed ratios after hydrolysis of Zileuton at pH 13 are 3.90, 4.02, and 4.13 at 82, 61, and 51°C, respectively.

Significant amounts of ketone V are formed in the pH range 1 to 5. Figure 4 shows that V results from hydrolysis of the oximes. Under acidic conditions the oximes hydrolyze to V, but they are relatively stable under neutral or basic conditions. More interestingly, Fig. 4 shows that the rate of hydrolysis of the oximes is not the same but somewhat faster for the less stable anti oxime. As the pH is increased, the anti oxime is converted into the more stable syn isomer. These observations are fully consistent with the kinetic model depicted in Scheme III, which was used to derive the time dependencies [Eqs. (3) to (9)] for the hydrolysis and/or interconversion of oximes through the common tetrahedral in-

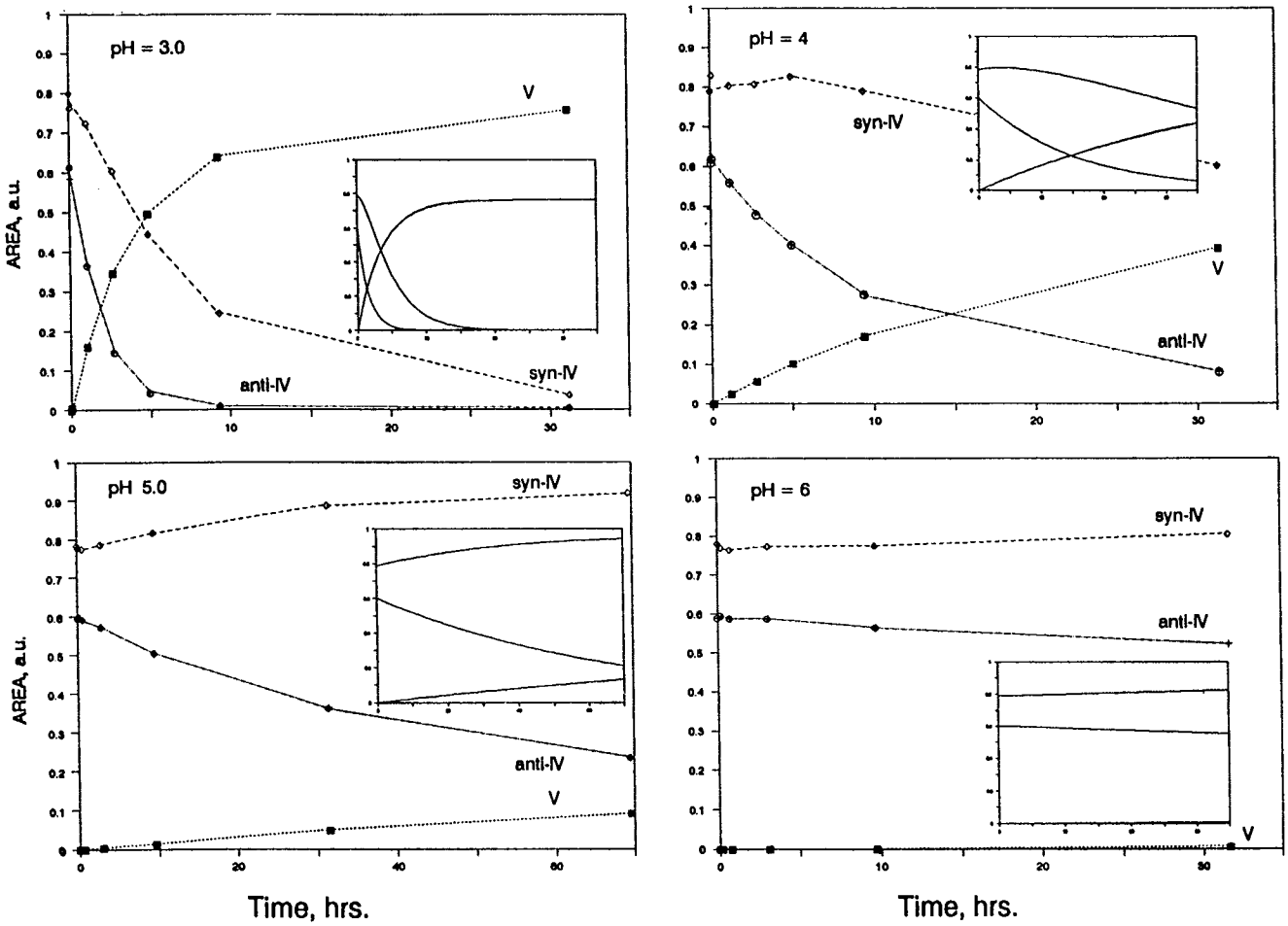
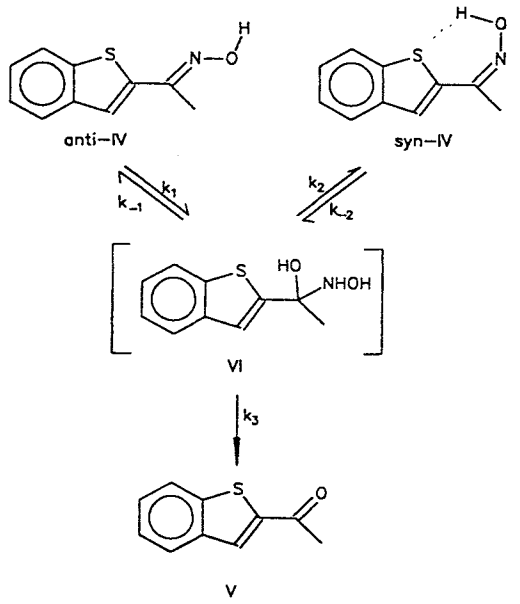


Fig. 4. Time dependence for the hydrolysis of syn- and anti-IV to V and for the syn- to anti-IV interconversion as a function of pH at 61°C. The insets are qualitative simulations of the actual data based on Eqs. (3)–(5) derived from the kinetic model depicted in Scheme IV. Citrate, 0.05 *M*; *I* = 0.2 *M*. Total oximes, initial concentration = 0.27 m*M*.



Scheme III

termediate VI. Equations (3) to (5) were used to generate simulated time profiles that correspond qualitatively with each of the observed profiles at the different pH values in the studies (insets in Fig. 4). At pH values below 4 the oxime nitrogen (pK_a ~4) is protonated, rendering a very electrophilic carbon that adds water easily to form the intermediate VI. This is also supported by noting that the amounts of V formed when the reaction is started with II is pH independent (data not shown) between 1 and 3 and then decays at pH 5.

$$[\text{syn-IV}] = \frac{(C_s - \text{syn-IV}_o * a)\exp(-at) - (C_s - \text{syn-IV}_o * b)\exp(-bt)}{b - a} \quad (3)$$

$$[\text{anti-IV}] = \frac{(C_a - \text{anti-IV}_o * a)\exp(-at) - (C_a - \text{anti-IV}_o * b)\exp(-bt)}{b - a} \quad (4)$$

$$[\text{ketone}] = V = \text{syn-IV}_o - \text{syn-IV} + \text{anti-IV}_o - \text{anti-IV} \quad (5)$$

where

$$C_s = \frac{\text{syn-IV}_o(k_{-1} + k_3)k_{-2} + k_{-1}k_{-2} * \text{anti-IV}_o}{k_{-1} + k_2 + k_3} \quad (6)$$

$$C_a = \frac{\text{anti-IV}_o(k_2 + k_3)k_1 + k_1k_2\text{syn-IV}_o}{k_{-1} + k_2 + k_3} \quad (7)$$

$$a + b = \frac{k_{-2}(k_{-1} + k_3) + k_1(k_2 + k_3)}{k_{-1} + k_2 + k_3} \quad (8)$$

and

$$a * b = k_1k_{-2}k_3/(k_{-1} + k_2 + k_3) \quad (9)$$

where syn-IV_o and anti-IV_o are the concentrations of syn and anti oximes at time 0 and k_i's the intrinsic rate constants indicated in Scheme III.

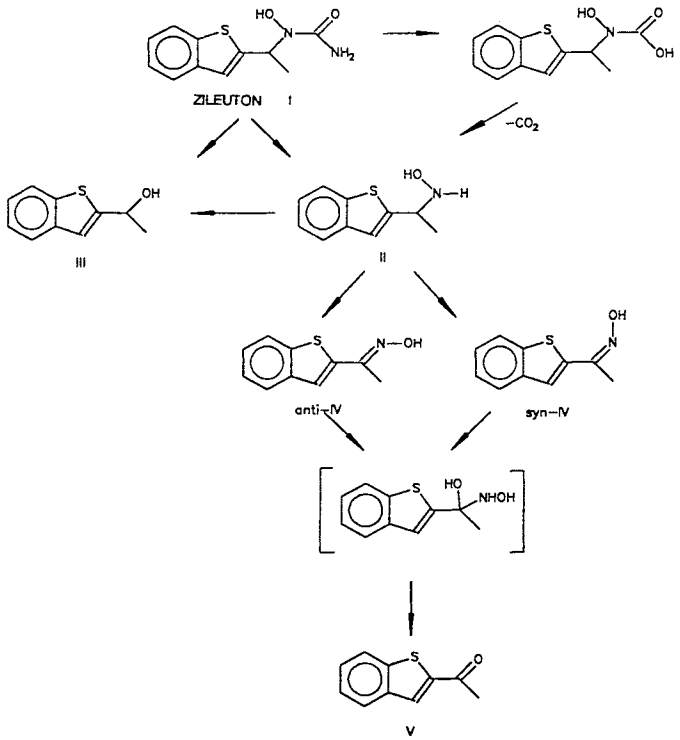
Table V. Effect of Oxygen on the Rate of Oxidation of Zileuton Hydroxylamine to Oximes IV at 25°C[a]

Condition	Time, min	% II	% IV
$+O_2$	0	100	0
	1	92	3.6
	30	15.3	87
	65	0.6	102
	126	0.4	99
$-O_2$	0	100	0
	1	96.6	0
	30	99.3	0
	66	102.5	0
	91	100.6	0.9

[a] [II] = 0.2 m*M*; carbonate, pH 9,0.05 *M*; *I* = 0.2 *M*.

Oxidation of Hydroxylamine to Oximes

Aliphatic hydroxylamines are air-oxidized to nitroso compounds which readily tautomerize to the more stable oxime form (17). An analysis of the products obtained from decomposition of II indicates that, with the exception of III formed directly from Zileuton at low pH, all the products observed during Zileuton degradation are derived from the primary decomposition product II. Degradation of II is pH independent in the acidic region and about twofold more stable than Zileuton under similar conditions (pH <4). Not surprisingly, II accumulates during Zileuton hydrolysis in acidic media (Figs. 2 and 3). At neutral and basic pH values II oxidizes rapidly to the two isomeric oximes, reaching a



Scheme IV

maximal decomposition rate at about pH 9. It was found that oxidation of II can be avoided by preventing exposure of the compound to molecular oxygen (Table V).

CONCLUSIONS

Scheme IV summarizes the decomposition pathway of Zileuton in aqueous solution. The observed products account, within experimental error, for all degraded drug. All these products can be separated and quantitated with the HPLC assay used and therefore provided alternative means to quantitate small decompositions of parent compound. Hydrolysis of the hydroxyurea group is the only important decomposition pathway in the pH range 1 to 13. Oxidation of III to the oximes and further hydrolysis of the oximes to V account for the most important decomposition products. Compounds able to complex to the *N*-hydroxyurea moiety, rendering a more electrophilic carbonyl carbon, can decrease the stability of Zileuton. The observed products provided some "historical" reference about the stress to which the parent drug might have been subjected.

ACKNOWLEDGMENTS

The helpful suggestions and comments provided by J. Summers, D. Brooks, S. Borodkin, R. Pyter, and B. Porter are gratefully acknowledged.

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