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Equilibria and kinetics of hydrolysis of ebifuramin (NSC-201047), an azomethine-containing structure exhibiting a reversible degradation step in acidic solutions

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Summary

During development of a parenteral formulation of ebifuramin (**I**, ethyl 4-hydroxy-2-(5-morpholinomethyl-2-oxazolidinyliminomethyl)furo[2,3b]pyridine-5-carboxylate), a sparingly soluble experimental cytotoxic agent, hydrolysis kinetics were found to be initial-concentration-dependent and to not follow first order kinetics. The present study was, therefore, carried out to evaluate the stability of ebifuramin at 37 °C at different acidic pH values and initial concentration. The primary route of degradation under acid conditions was found to involve equilibrium hydrolysis of an azomethine bond with subsequent apparent first-order degradation of at least one of the initial hydrolysis products. Forward and reverse rate constants and the equilibrium constant for the primary reversible step, as well as pseudo-first-order rate constants for the secondary hydrolysis, were determined. One of the primary degradation products was isolated and characterized. The sites of protonation-deprotonation equilibria in aqueous solution were defined by pK_a determinations of ebifuramin and model compounds.

Introduction

Hydrolysis kinetics, at acidic pH values, of ebifuramin (**I**, NSC-201047, ethyl 4-hydroxy-2-(5-morpholinomethyl-2-oxazolidinyliminomethyl(furo[2,3b]pyridine-5-carboxylate), a sparingly soluble experimental cytotoxic agent (Snyder and Ebetino, 1966; J. Cradock, National Cancer Institute, personal communications), were found to be initial-concentration-dependent and to not follow first order kinetics. For example, at room temperature, a 1 : 10 dilution (5 mg/ml) with D5W of an acidic experimental formulation of ebifuramin, had a t_{90} of $\approx$ 280 h, whereas for a 1 : 100 dilution (0.5 mg/ml), the t_{90} was $\approx$ 112 h. These differences could not be attributed to pH differences. The present study was, therefore, carried out to evaluate the stability of ebifuramin at 37 °C at differing acidic pH values and initial concentrations in an attempt to understand its unusual hydrolysis kinetics.

Ebifuramin is structurally related to furazolidone, nitrofurantoin and furaltadone (Harris, 1963) which have shown antibacterial activity (Kessler et al., 1976). All of these compounds contain an azomethine bond (–N = CH–). The

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$$A \underset{k_r}{\overset{k_f}{\rightleftharpoons}} B + C$$

Scheme 1

degradation of azomethine bond-containing drugs in aqueous solutions has been reported previously. The hydrolysis of diazepam (Nakano et al., 1981; Connors et al., 1986) and nitrofurantoin (Inotsume and Nakano, 1981; Connors et al., 1986) in acidic solutions, involve reversible azomethine bond cleavage presumably through a mechanism involving reversible addition of water across the azomethine bond to form a transient carbinolamine which then rearranges to the product(s). Hydrolysis of nitrofurantoin initially results in the formation of 5-nitrofurfural and 1-aminohydantoin, whereas hydrolysis of the azomethine bond in diazepam results in opening of the 7-membered azepine ring. The hydrolysis of nitrofurantoin can be described by Scheme 1 where the forward reaction is pseudo-first-order, while the reverse reaction is pseudo-second-order.

The overall position of the equilibrium, K_{eq}, is defined by the ratio, k_f/k_r. Several methods may be proposed to isolate k_f and k_r. Provided that the reaction is followed to equilibrium, k_f and k_r may be extracted from the time-dependent disappearance of the reactant, A, using Eqn. 1 (Moore and Pearson, 1981):

$$\ln[ax_e + x(a - x_e)]/[a(x_e - x)] = k_f[(2a - x_e)/x_e]t \quad (1)$$

where a is the initial concentration of the reactant A, x is the extent of reaction at any time, t, and x_e is the extent of reaction at equilibrium. A plot of the left hand side of Eqn. 1 vs time should be linear with a zero intercept and a slope of $k_f(2a - x_e)/x_e$. The value for k_r is then calculated from Eqn. 2.

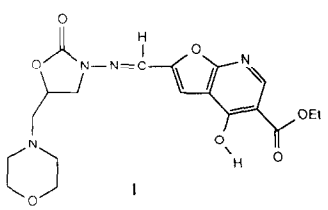
$$k_r = k_f(a - x_e)/x_e^2 = k_f/K_{eq} \quad (2)$$

Other methods for isolation of k_f and k_r include (i) initial rate methods, in which the rate of appearance of a product is used to estimate k_f; or (ii) the use of a trapping agent, whereby one of the products (B or C) is trapped by an excess reagent, thus preventing the reverse reaction from occurring. This converts Scheme 1 into a reaction which follows pseudo-first-order kinetics, with a rate constant equal to k_f.

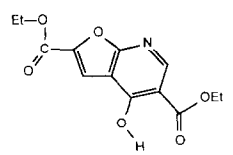
Materials and Methods

Materials

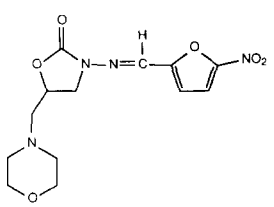
Ebifuramin (ethyl 4-hydroxy-2-(5-morpholinomethyl-2-oxazolidinyliminomethyl(furo[2,3b]-pyridine-5-carboxylate; NSC-201047; **I**; lot no. 9279-055A), diethyl-4-hydroxyfuro[2,3b]pyridine-2,5-dicarboxylate (**II**; lot no. 1372-A-93) and (+)-(R)-5-morpholinomethyl-3-(5-nitrofurfurylideneamino)-oxazolidin-2-one (**III**; lot no. 1197-HS-62) were used as received from the National Cancer Institute, Bethesda, MD, U.S.A. (supplied by Norwich-Eaton Pharmaceuticals, Inc., Norwich, NY 13815). All reagents were ACS reagent grade or better and were used without further purification. Organic solvents were HPLC grade. Water was deionized, then glass-distilled (Mega-Pure System Model MP-1, Corning).



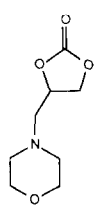
I



II



III



IV

Equipment

Proton (^1H) and carbon (^{13}C) FT-NMR spectra were obtained for deuteriochloroform ($CDCl_3$) (Stohler, 99.8% D) and hexadeuteriodimethylsulfoxide ($(CD_3)_2SO$) (Aldrich, 99.9% D) solutions with a Varian XL-300 spectrometer. Electron impact (70 eV) mass spectrometry (EIMS) was performed at high resolution with a Ribermag R10-10 C mass spectrometer. FT-IR was performed with an IBM IR-30 series spectrophotometer. Solid samples were carefully incorporated into finely powdered dry KBr and compressed, while liquid samples were examined as a thin film on a KBr window.

HPLC Analysis

Analysis of ebifuramin and its decomposition products used an Altex 110A pump, a Rheodyne 7125 injector (20 μl loop), a 15×0.46 cm reverse phase column packed with 5 μm ODS-Hypersil (Shandon), and a Waters 440 UV detector (254 nm). The mobile phase was 15% acetonitrile, 5% tetrahydrofuran and 80% 0.01 M dibutylamine phosphate (pH adjusted to 2.6 with phosphoric acid) at a flow rate of 1.5 ml/min. Calibration plots of peak area vs concentration of **I** (Shimadzu C-R3A integrator) were linear over the concentration range 1-200 μg/ml and passed through the origin.

Thin layer chromatography (TLC)

TLC was performed on silica gel plates (EM Reagents Silica Gel 60 F_{254}, Darmstadt, F.R.G.), with a solvent system consisting of methanol-chloroform (10 : 90). Detection was by UV illumination (254 and 366 nm).

Synthesis of (±)-4-morpholinomethyl-1,3-dioxolan-2-one (IV)

IV ($C_8H_{13}NO_4$) was synthesized by cyclization of 3-morpholino-1,2-propanediol (Aldrich) with *N,N'*-carbonyldiimidazole (Aldrich) in dry toluene (Kutney and Ratcliffe, 1975). A high-resolution EIMS of the product displayed a molecular ion peak with exact mass m/e = 187.08, corresponding to $C_8H_{13}NO_4$. FT-IR spectra demonstrated the appearance of a band at 1820 cm^{-1} (cyclic carbonate C = O stretch) and the absence of a band at 3400 cm^{-1} (OH stretch). ^1H and ^{13}C FT-NMR spectra were consistent with the structure given for **IV**.

Isolation of the primary hydrolysis product (V) of ebifuramin

Ebifuramin (500 mg), methanesulfonic acid (10 equivalents) and formaldehyde (10^4 equivalents) were dissolved in 50 ml of water. The solution was stirred for 2 days. The resulting precipitate was filtered (5–15 μm sintered glass), then redissolved with $CHCl_3$ and passed through the filter. The $CHCl_3$ solution was then evaporated and the residue was dried to yield 221 mg of a pale yellow powder (79% of theoretical). A single spot was obtained on TLC with a larger R_f value than that of ebifuramin. HPLC of this material, dissolved in the mobile phase used for the HPLC analysis for ebifuramin, gave a peak ($V_r = 21.5$ ml) which accounted for 99.4% of the total absorbance of the material eluted from the column. Calibration plots of peak area vs concentration of **V** (Shimadzu C-R3A integrator) were linear over the concentration range 0.625–12.5 μg/ml and passed through the origin. The product had m.p. 147.0–147.5 °C with decomposition. An FT-IR spectrum was consistent with **V** (aldehyde C = O stretch, 1697 cm^{-1}; ethyl ester C = O stretch, 1673 cm^{-1}; absence of an oxazolidinone C = O stretch, 1759 cm^{-1}). A high resolution EIMS displayed a molecular ion peak with exact mass m/e = 235.0485, corresponding to $C_{11}H_9NO_5$. Elemental microanalysis gave the following results: %C, 56.26; %H, 3.96; %N, 5.97. Expected for $C_{11}H_9NO_5$: %C, 56.18; %H, 3.86; %N, 5.96. Fourier transform NMR (^1H and ^{13}C) spectra were also obtained for V. The NMR data were consistent with the structure given, ethyl 4-hydroxy-(2-formyl)furo[2,3b]pyridine-5-carboxylate (**V**).

Spectrophotometric and potentiometric pK_a values

These were obtained by standard methods (Albert and Serjeant, 1971). UV-vis spectra (200–500 nm) were obtained with HP8451A Diode Array or Shimadzu UV-260 spectrophotometers. pH values were measured with Fisher Accumet 610A or Brinkman Metrohm 632 pH meters. The following buffer systems ($\mu = 0.1$ M; NaCl or KCl) were employed for pK_a determinations: HCl, (0.1 M

and 0.01 M), sodium acetate (pH 4.0–5.4), potassium succinate (pH 5.9) sodium phosphate (pH 6.9, 7.5) and Tris-HCl (pH 8.0). Aliquots (25 μl) of stock solutions (2.4×10^{-2} M) of **I** and **III** (in dilute methanesulfonic acid) and **II** (in 95% ethanol) were diluted to 25.0 ml immediately before spectra were determined. Time-dependent observations showed that no significant degradation had occurred during measurements. **IV** was titrated with standard HCl (Fisher Certified) at an ionic strength of 0.1 M.

Kinetics of hydrolysis

The decomposition of ebifuramin was studied in the following aqueous solutions ($\mu = 0.1$ M; NaCl) at 37°C: methanesulfonic acid (0.1 and 0.01 M), sodium formate buffer (pH 3.3) and sodium acetate buffer (pH 4.0 and 5.0). Two series of experiments were performed. The effect of initial **I** concentrations ranging from 25 μg/ml to 200 μg/ml on the kinetics of hydrolysis was studied in 0.1 M methanesulfonic acid solutions. The effect of pH (from 1 to 5) on the kinetics was studied at an initial concentration of 25 μg/ml (5.975×10^{-5} M), both in the presence and absence of 6×10^{-1} M formaldehyde. pH values were measured with a Fisher Accumet 610A pH Meter. The pH meter was calibrated against standard buffers. Loss of ebifuramin and the appearance of degradation products was followed by HPLC. The following retention volumes were seen: 6.9–7.5 ml (ebifuramin, **I**); 12.2–12.7 ml (secondary degradation product); 21–22 ml (primary degradation product, **V**).

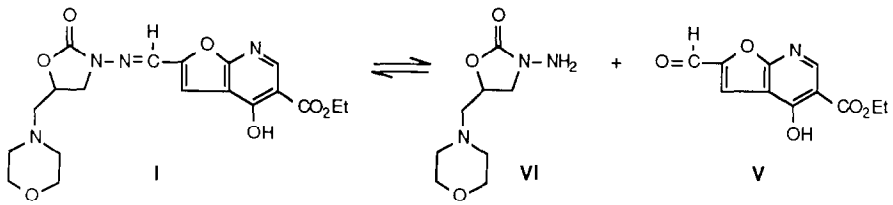
Results and Discussion

Semilogarithmic plots against time for the loss of ebifuramin under acidic pH conditions were biexponential. Inspection of the structure of ebifuramin reveals a number of possible sites of protonation and degradation pathways.

pKa values for ebifuramin in water

Spectrophotometer determination of the pK_a of **I** gave a value of 5.24 ± 0.04 at 25°C and 5.16 ± 0.03 at 37°C. **II** gave a pK_a value range of 4.85–5.08 (25°C) depending on the wavelength of analysis. **III** showed no appreciable spectral changes over the pH range of 2–8 while **IV** showed a potentiometrically determined pK_a value of 5.29 ± 0.04 at 25°C. Comparison of the spectral changes for **I** with **II** and **III** indicated that ebifuramin more closely paralleled **II** rather than **III**. This suggested that the ionization observed for ebifuramin involved the hydroxyl proton, rather than protonation-deprotonation at the azomethine nitrogen. The pK_a for protonation-deprotonation at azomethine nitrogens has been reported to have values in the range 3.3–3.5 (Connors et al., 1986). Protonation of the pyridine nitrogen of **I** and **II** was not expected to occur in aqueous solutions, as this nitrogen is very weakly basic (Acheson, 1967). Snyder and Ebetino (1966) claimed on the basis of solid-state IR spectra, that **I** is a 4-hydroxypyridine, rather than the more commonly observed 4-pyridone (Acheson, 1967). In aqueous solutions of **I**, however, it is possible that tautomerization occurs to give the 4-pyridone. The data in the present work is not sufficient to distinguish between these possibilities. The temperature dependence of the pK_a for ebifuramin was shown to be small (0.08) between 25°C and 37°C. This is further support for assigning the observed spectral changes with pH to ionization of the hydroxyl proton, rather than to protonation of the –N = CH– group.

The morpholino nitrogen of **I** was expected to be involved in acid–base equilibria in the pH range 2–8. However, the protonated and unprotonated forms were not expected to display significantly different UV-VIS spectra. The pK_a for morpholino group in **I** was estimated by potentiometric titration of the model compound **IV**. The weak basicity of this group compared to *N*-ethylmorpholine, $pK_a = 7.67$ (Perrin, 1965) probably results from the inductive effect of the cyclic carbonate (1,3-dioxolan-2-one) group. A very small change in the spectral properties of **I** was observed on comparing solutions of pH 1 and 2. These changes included a slight shift in the absorption bands to shorter wavelengths, suggesting increased electron localization. This might be expected to occur on protonation of the azomethine nitrogen.



Scheme 2

The pK_a value for this group is probably 2 or below.

Hydrolysis of ebifuramin

Based on the structural similarity to nitrofurantoin (Inotsume and Nakano, 1981), the primary site of initial reaction in acid media was expected to be equilibrium hydrolysis of the azomethine bond to give the aldehyde (**V**) and the 3-amino-oxazolidin-2-one (**VI**), Scheme 2. **V** was isolated and characterized (see Materials and Methods) as one of the initial degradation products. Attempted isolation of **VI** was not successful.

The concentration-dependent hydrolysis of ebifuramin was studied in 0.1 M methanesulfonic acid. Methanesulfonic acid was used in place of the more usual hydrochloric acid as the hydrochloride salt of ebifuramin has been reported to have limited aqueous solubility (A. Hussain, University of Kentucky, unpublished results). HPLC chromatograms of dilute ebifuramin solutions, on storage, showed that the peak corresponding to ebifuramin decreased in area with time. Simultaneously, a late eluting peak ($V_r = 21.5$ ml, corresponding to **V**) appeared, reached a maximum, and then disappeared with the formation of a second peak ($V_r = 12.2$ ml). Other minor changes occurred in the chromatograms near the solvent front at later time points. Fig. 1 shows the change in ebifuramin concentration with time from 25, 100 and 200 μg/ml solutions, while Fig. 2 shows the change in concentration with time of the primary degradation product, **V**. The concentrations of **I** and **V** were calculated from the peak areas in the HPLC chromatograms and standard curves.

The plots in Fig. 1 and 2 are interpreted in terms of a rapid reversible equilibrium reaction leading to a pseudo-equilibrium, followed by a slower reaction which obeys apparent pseudo-first-order kinetics. The apparent first-order rate constants, k, from the slopes of the terminal phases

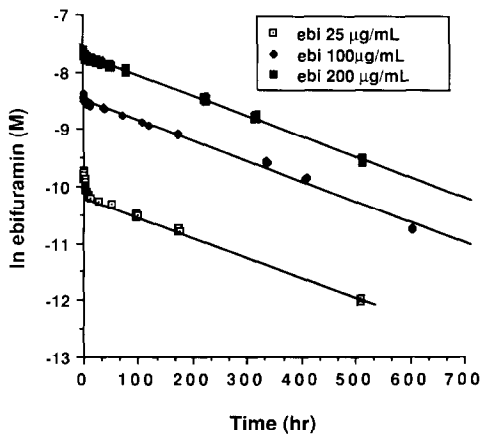


Fig. 1. Time-dependent changes in ln molar concentration of ebifuramin as a function of initial ebifuramin concentration at 37 °C in 0.1 M methanesulfonic acid.

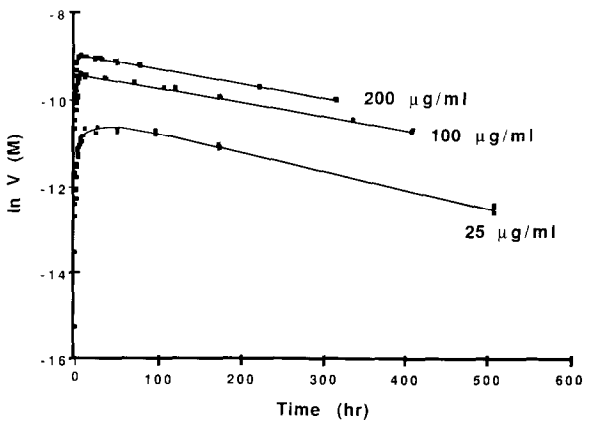


Fig. 2. Time-dependent appearance of the primary degradation product of ebifuramin, **V**, as a function of initial ebifuramin concentration at 37 °C in 0.1 M methanesulfonic acid.

of Fig. 1 and 2 are reported in Table 1. Also reported are the percentages of ebifuramin at zero time from the extrapolated terminal phases and the equilibrium constants, K_{eq}, calculated according to Eqn. 2.

As would be expected for a reversible reaction described by Scheme 1, the initial phase of the curves in Fig. 1 indicate that the equilibrium dissociation of ebifuramin to **V** and **VI** is more complete as the initial concentration of ebifuramin is decreased. The difference in the value for K_{eq} (Table 1) at an initial concentration of 200 μg/ml, compared to the lower initial concentrations, may be a real function of the higher initial concentration. Alternatively, it may simply reflect the greater difficulty of obtaining reliably measured concentration differences where the change in concentration was small. A 50 mg/ml solution of ebifuramin at the same pH did not appear to dissociate and hydrolyzed according to apparent first-order kinetics.

The slower terminal phase of the time-dependent loss of ebifuramin is due to the apparent first-order hydrolysis of one or both of the primary degradation products as well as ebifuramin itself; the most likely reaction being hydrolysis of the ethyl ester function in both **I** and **V**. It is noteworthy that the values for the terminal slopes for loss of **I** are very close numerically to those for loss of **V**, and are nearly independent of the initial drug concentrations (Table 1). Hydrolysis of **V** in 0.1 M methane sulfonic acid followed apparent first order kinetics with a half-life essentially identical to that of the terminal slopes for the loss of **I**. Since the concentrations of **I** and **V** in solution

TABLE 1

*Apparent first-order terminal rate and equilibrium constants for hydrolysis of **I** and **V** in 0.1 M methane sulfonic acid at 37 °C with changing initial ebifuramin concentrations*

Initial conc. (μg/ml)	$k \times 10^3$ (h^{-1}) (from loss of **I**)	$k \times 10^3$ (h^{-1}) (from loss of **V**)	% **I** at t_0 (extrapolated)	K_{eq} ($M \times 10^5$)
25	3.65	4.05	66.2	1.04
100	3.12	3.24	81.5	1.01
200	3.34	3.32	89.3	0.63

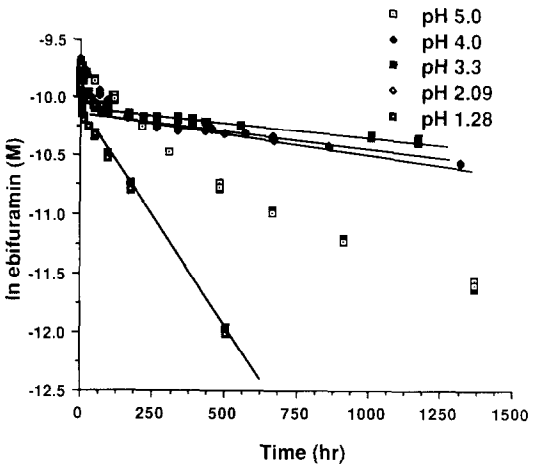
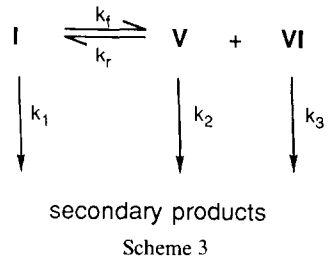


Fig. 3. Time-dependent loss of ebifuramin at 37 °C and various pH values for an initial ebifuramin concentration of 25 μg/ml.

will vary with initial concentration, the terminal slopes should have varied with changes in the concentration if only one of the species underwent secondary hydrolysis. The fact that the slopes appear to be nearly independent of the initial drug concentration suggests that ebifuramin and at least one of the primary degradation products, **V** and **VI**, degrade at comparable rates. A priori, the results are more consistent with ester hydrolysis being the secondary reaction.

The hydrolysis of ebifuramin, 25 μg/ml, was investigated in the pH range 1–5. Plots of the time-dependent concentrations of **I** (Fig. 3) at pH values in the range of 1–4 were very similar to those seen in Fig. 1, i.e., they did not follow first-order kinetics. At pH 5.04, the reaction seemed to take much longer to reach a pseudo-equilibrium, possibly indicating that subsequent reactions were occurring in the same time range as the approach to pseudo-equilibrium. As indicated by the methanesulfonic acid data, a more realistic scheme for the overall disappearance of ebifuramin would be given by Scheme 3 rather than Scheme 2. Based on the data from Fig. 1, it is reasonable to assume that at least **I** and **V** undergo reaction and that the reactions are first-order; confirmed when **V** was studied independently.

One way to simplify the analysis of such a complex reaction scheme is through the use of a



Scheme 3

trapping agent. For example, the reaction defined by k_r in Scheme 3 presumably involves the reaction of **V**, an aldehyde, with the amino group of **VI**. If an excess of an aldehyde, other than **V**, was placed in the solution, effectively removing **VI** from solution, the reverse reaction in Scheme 3 effectively could not occur. Therefore, the overall disappearance of ebifuramin should follow pseudo-first-order kinetics with a rate constant equivalent to k_f. In the present study formaldehyde was chosen as the trapping aldehyde. In the presence of 0.6 M formaldehyde and at pH values in the range of 1–5, the disappearance of **I** obeyed pseudo-first-order kinetics over greater than two half-lives. The apparent rate constants in the presence of excess formaldehyde, k_f', as a function of pH are given in Fig. 4.

Analytical solutions of the kinetic rate and equilibrium expressions for the reaction described by Scheme 3 would be very difficult. However, if the reaction is thought of as occurring in two

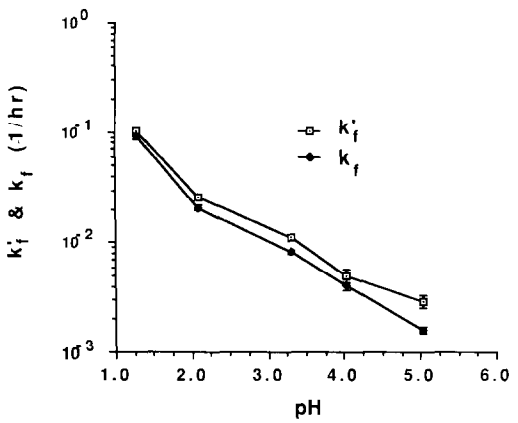


Fig. 4. Dependence of k_f and k_f' on pH for the hydrolysis of ebifuramin at 37 °C and an initial ebifuramin concentration of 25 μg/ml.

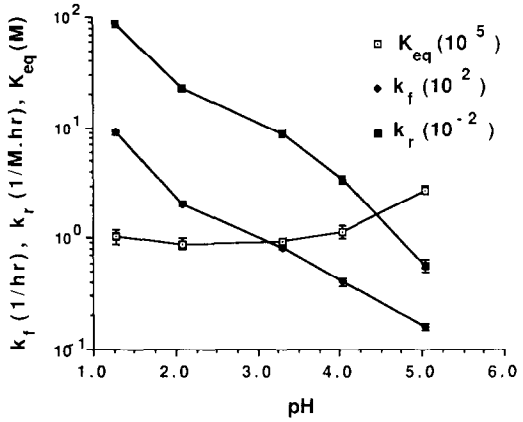


Fig. 5. Dependence of k_f, k_r and K_{eq} on pH for the hydrolysis of ebifuramin at 37 °C and an initial ebifuramin concentration of 25 μg/ml.

phases, an early equilibrium phase and a secondary reaction phase, it should be possible to correct for the loss of ebifuramin due to secondary reactions during the equilibrium phase using Eqn. 3:

$$\ln[\text{ebifuramin}]_{corr} = \ln[\text{ebifuramin}]_t + kt \qquad (3)$$

where k is the observed slope of the linear terminal phase of the plot of ln $[\text{ebifuramin}]_t$ versus time, t, at each pH. The corrected ebifuramin concentrations were then employed in Eq. 1. The pH dependency of the forward and reverse rate constants obtained from Eqns. 1 and 2 are shown in Figs. 4 and 5. In all cases, the plots of the left hand side of Eqn. 1 vs time were linear with correlation coefficients of 0.991 or greater. The plots were all initially fitted with finite y-intercept values, which were found by t- and F-tests to not be statistically different from zero. The plots were then forced through zero to give the data plotted in Figs. 4 and 5. The coefficients for the linear plots were significant at very high levels of probability (99.999% or greater). The values for k_f from the fit of the data to Eqn. 1 are within 15–25% of the values from the formaldehyde experiments, k_f' at corresponding pH values except at pH 5, for which the deviation is about 50%. However, as stated earlier, the assumption that a pseudo-equilibrium had been established at pH 5

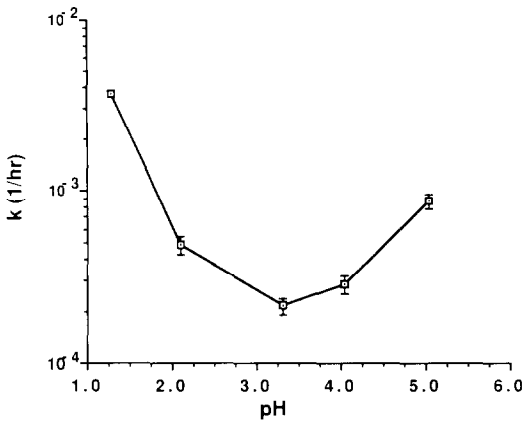


Fig. 6. Dependence of k (from terminal slopes) on pH for the hydrolysis of ebifuramin at 37 °C and an initial ebifuramin concentration of 25 μg/ml.

may be incorrect. The small differences between k_f and k'_f may be due to a solvent or medium effect from the relatively high concentration (0.6 M) of formaldehyde used in the trapping experiments. The pH dependency of K_{eq} is also shown in Fig. 5.

The pH-rate profiles for k_f, k'_f, k_r, K_{eq} and k, shown in Figs. 4–6, are not easily interpreted. For the semilog plots of k_f, k_r and k'_f vs pH (Figs. 4–5), the slope below pH 2 approaches negative unity. This may be ascribed to increased protonation of the azomethine nitrogen (see earlier pK_a discussion). The lack of a simple relationship between pH and the rate constants, k_f, k_r and k'_r for the pH range 2–5 probably results from the multiple equilibria for **I** at the upper end of this pH range. It may also indicate that the pK_a of the carbinolamine formed on hydration of the azomethine bond may play a role. The equilibrium constant, K_{eq}, is virtually independent of pH up to pH 4. In the semilog plot of k (terminal rate constant) vs pH (Fig. 6), the slope between pH 1 and 2 is close to negative unity, suggesting that the secondary reaction(s) are subject to an acid catalysis. Similarly, the increase in k as the pH is raised above 4 suggests that the secondary reaction(s) may be subject to base catalysis at higher pH values. The shape of the pH–rate profile for this secondary reaction is consistent with the proposed ester hydrolysis pathway.

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References

Acheson, R.M., *An Introduction to the Chemistry of Heterocyclic Compounds*, 2nd edn., Wiley, New York, 1967, pp. 218–222.

Albert, A., and Serjeant, E.P., *The Determination of Ionization Constants*, Chapman and Hall, London, 1971, pp. 14–38, 70–101.

Connors, K.A., Amidon, G.L. and Stella, V.J. (Eds.), *Chemical Stability of Pharmaceuticals*, 2nd edn., Wiley, New York, 1986, pp. 412–419, 621–624.

Harris, N.D., Stereospecific syntheses of some optically active 5-substituted 3-aralkylideneamino-2-oxazolidinones. *J. Org. Chem.*, 28 (1963) 745–748.

Inotsume, N., and Nakano, M., Reversible azomethine bond cleavage of nitrofurantoin in acidic solutions at body temperature. *Int. J. Pharm.*, 8 (1981) 111–119.

Kessler, H.-J., Rufer, C. and Schwarz, K., Chemotherapeutische Nitroheterocyclen XXI. *Eur. J. Med. Chem.*, 11 (1976) 19–23.

Kutney, J.P. and Ratcliffe, A.H., A novel and mild procedure for preparation of cyclic carbonates. *Synth. Comm.*, 5 (1975) 47–52.

Moore, J.W. and Pearson, R.G., *Kinetics and Mechanism*, 3rd edn., Wiley, New York, 1981, pp. 304–305.

Nakano, M., Inotsume, N., Kohri, N. and Arita, T., Reversible ring opening reaction of diazepam in acid media around body temperature. *Int. J. Pharm.*, 3 (1981) 195–204.

Perrin, D.D., *Dissociation Constants of Organic Bases in Aqueous Solution*, Butterworths, London, 1965, no. 1454.

Snyder, H.R. and Ebetino, F.F., A new synthesis of furo[2,3-b]pyridine derivatives. *J. Heterocyclic Chem.*, 3 (1966) 202–5.