International Journal of Pharmaceutics, 78 (1992) 59–67

IJP 02597

Kinetics for degradation of rifampicin, an azomethine-containing drug which exhibits reversible hydrolysis in acidic solutions

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(Received 22 April 1991)
(Modified version received 29 July 1991)
(Accepted 6 August 1991)

Key words: Rifampicin; Azomethine; Hydrolysis; Reversible reaction; Kinetics; pH-rate profile

Summary

The degradation of rifampicin (**I**) was studied at six pH values from pH 1 to 5 (37°C, $I = 0.1$ M). The principal reaction under these conditions is reversible hydrolysis of an azomethine bond. Oxidative side-reactions were prevented by the addition of ascorbic acid to the reaction media. Concentration-time plots were initially concentration dependent and approached an equilibrium, consistent with a reversible reaction. Studies were performed at low initial drug concentration (21 μg/ml), as the primary reaction product was observed to precipitate at higher substrate concentrations. Apparent rate constants for hydrolysis were calculated from the concentration-time data by two methods. The first assumed pseudo-first order irreversible behavior (forward reaction only), as linear log concentration-time plots were observed for about three half-lives. The second attempted to account for the expected pseudo-second order reverse reaction as well. The rate constants were in close agreement, due to the low initial drug concentration and the very low concentration of **I** (> 1–7.5% of initial) at equilibrium. The pH-rate profile appears complex below pH 4.3, but displays a slope of −0.97 between pH 4.3 and 5.0, suggesting specific acid catalysis, or a kinetic equivalent.

Introduction

Rifampicin (rifampin, 3-(4-methyl-1-piperazinyliminomethyl)rifamycin; **I**) is an anti-tubercular drug which has been in clinical use for some 20 years. Although a quantitative study of rifampicin hydrolytic stability as a function of pH has been previously reported (Seydel, 1970), it was confined to a very narrow pH range (0.75–1.75). This early study indicated limited stability in acid media. The principal degradation products in the absence of oxidative reactions are 3-formylrifamycin SV (**II**) and 1-amino-4-methyl-piperazine (**III**). Exposure to alkaline pH values results in a further degradation product, which is also the major metabolite in humans, 25-desacetylrifampicin (Ratti et al., 1981). In view of the exposure of this drug in oral dosage formulations to varying acidic conditions in the stomach and duodenum, it was of interest to examine the stability of rifampicin over a wider range of acidic pH values.

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$$\mathrm{I} + \mathrm{H_2O} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} \mathrm{II} + \mathrm{III}$$

Scheme 1.

Rifampicin, as well as a number of other drugs, such as ebifuramin (Snyder and Ebetino, 1966), diazepam, furazolidone, nitrofurantoin and furaltadone all contain an azomethine bond (–N=CH–). Studies of the kinetics of degradation of such azomethine bond-containing drugs in aqueous solutions have been reported previously. The hydrolyses of diazepam (Han et al., 1977; Nakano et al., 1981; Connors et al., 1986a), nitrofurantoin (Inotsume and Nakano, 1981; Connors et al., 1986b) and ebifuramin (Prankerd and Stella, 1989) in acidic solutions all involve reversible azomethine bond cleavage. Reversible addition of water across the azomethine bond to form a transient carbinolamine, which then rearranges to the product(s), is the most likely mechanism for this reaction (Prankerd and Stella, 1989). The hydrolyses of nitrofurantoin and ebifuramin can be described by Scheme 1, where the forward reaction is pseudo-first order (k_f), while the reverse reaction is pseudo-second order (k_r) (Prankerd and Stella, 1989).

The overall position of the equilibrium (K_{eq}) is defined by the ratio (k_f/k_r). For Scheme 1, the second order reverse reaction complicates the estimation of rate constants. Treatment of experimental kinetic data according to Scheme 1 was recently discussed by Prankerd and Stella (1989). The following expression (Eqn 1) (Moore and Pearson, 1981) was used to estimate k_f and k_r for ebifuramin over the pH range 1–5 from the time course of its disappearance:

$$\ln \frac{ax_e + x(a - x_e)}{a(x_e - x)} = k_f \frac{2a - x_e}{x_e} t \qquad (1)$$

where a is the initial concentration of the reactant, x is the molar extent of reaction at any time (t), and x_e is the molar 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 may then be calculated from Eqn 2:

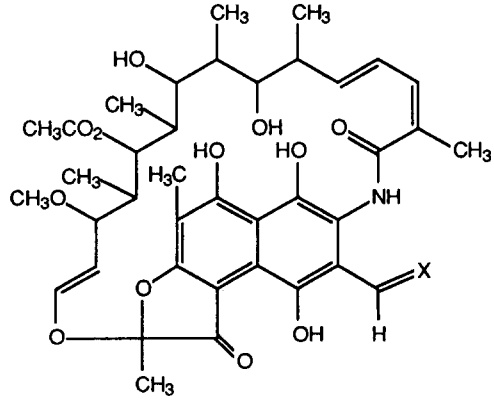
$$k_r = k_f \frac{a - x_e}{x_e^2} = \frac{k_f}{K_{eq}} \qquad (2)$$

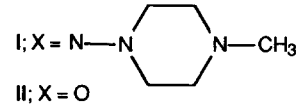
Other methods for isolation of k_f, such as the use of trapping reagents, have been discussed by Prankerd and Stella (1989).

Experimental

Materials

Rifampicin (3-(4-methyl-1-piperazinyliminomethyl)rifamycin) (**I**) (Ciba-Geigy lot no. 37960; Merrell-Dow MDL 13,407) and 3-formylrifamycin SV (**II**) (Merrell-Dow MDL 29,038) were used as





received. All reagents were ACS reagent grade or better and were used without further purification. Acetonitrile was pre-filtered HPLC grade (Fisher). Institutional deionized water was glass-distilled (Mega-Pure System Model AG-3, Corning).

Methods

Isocratic high-performance liquid chromatography (HPLC) was used for all analyses. Analysis of rifampicin and its decomposition product used an LDC CM4000 pump, a Rheodyne 7125 injector (20 μl loop), a 150 × 4.6 mm reversed-phase column (Nucleosil C8, 5 μm; Keystone) and an LDC SM3100 UV detector (255 nm). The mobile phase was 38.5% acetonitrile, 5% water and 46.5% 0.05 M Tris-HCl buffer (pH 7.0) v/v at a flow rate of 1 ml/min. A plot of peak area (Shimadzu C-R3A integrator) vs concentration of **I** was linear ($r^2 = 0.999$) in the range 0.050–200 μg/ml and passed through the origin. The coefficient of variation was 0.66% at 10 μg/ml ($n = 3$) and 3.1% at 1.67 μg/ml ($n = 10$). A plot of peak area vs concentration of **II** was linear ($r^2 = 0.999$) in the range 0.050–27.5 μg/ml and the coefficient of variation was 0.75% at 5.5 μg/ml ($n = 8$).

The time course for rifampicin decomposition was studied in the following aqueous solutions (ionic strength adjusted to 0.1 M with NaCl, where needed) at 37.0 ± 0.05°C: hydrochloric acid (0.1 and 0.01 M) and sodium chloroacetate (0.1 M; pH 2.8), sodium formate (0.1 M; pH 3.4) and sodium acetate (0.1 M; pH 4.3 and 5.0) buffers. The initial drug concentration was approx. 21 μg/ml (2.6×10^{-5} M). All solutions contained ascorbic acid (100 μg/ml) to prevent known oxidative side-reactions. pH values were measured with a Beckman Model 4500 Digital pH Meter, calibrated against phosphate (pH = 6.98 at 37°C) and phthalate (pH = 4.02 at 37°C) pH standard solutions (Fisher). Temperature control was maintained with a Haake NK22 water bath. A stock solution of approx. 2.1 mg rifampicin (Cahn Model 2000 Electrobalance) in 1.0 ml of acetonitrile was freshly prepared each day. 100 μl of the stock solution was then added at zero time to 10.0 ml of the reaction solution (pre-equilibrated at 37.0 °C), mixed thoroughly, a zero time aliquot taken for analysis, and the reaction flask then returned to the bath. The reaction solutions had an acetonitrile content of 0.99% v/v. pH values measured at the beginning and end of the experimental runs did not differ by more than 0.04 pH unit. Trapping experiments were performed by repeating the above reaction (at pH 2.8) in a reaction solution that was also 1.0 M in formaldehyde. Loss of **I** and the appearance of degradation products was followed by the HPLC method described above. Instrumental drift was checked with an external standard. The concentrations of **I** were calculated from the peak areas in the HPLC chromatograms and a standard curve. The following retention volumes (V_r) were seen: 5.9–6.1 ml (rifampicin); 9.9–10.1 ml (primary degradation product); 9.9–10.1 ml (authentic 3-formylrifamycin SV, **II**). All kinetic runs were performed in duplicate at least.

Results and Discussion

HPLC chromatograms of reaction solutions showed that the peak corresponding to **I** decreased in area with time, while a second peak (**II**) appeared. The appearance of 1-amino-4-methylpiperazine (**III**) could not be followed by this method, due to its polarity and UV transparency, although changes were seen at the solvent front. An additional peak was seen in the absence of an antioxidant, presumably rifampicin quinone (**IV**). Formation of **IV** had not previously been reported in acid solutions. Initial concentrations of rifampicin had to be low (≤ 21 μg/ml), due to the very low water solubility of the primary degradation product (**II**). At initial concentrations greater than 21 μg/ml, precipitation occurred before equilibrium was reached for the first decomposition step (initial phase of the biexponential semilog plot). Although such precipitation would not invalidate kinetic analysis based on irreversible apparent first-order kinetics, it would invalidate calculations based on reversible kinetics, as an equilibrium constant describing the saturated solubility of **II** would be required. The formation of **II** during decomposition of rifampicin in acid solutions has been reported pre-

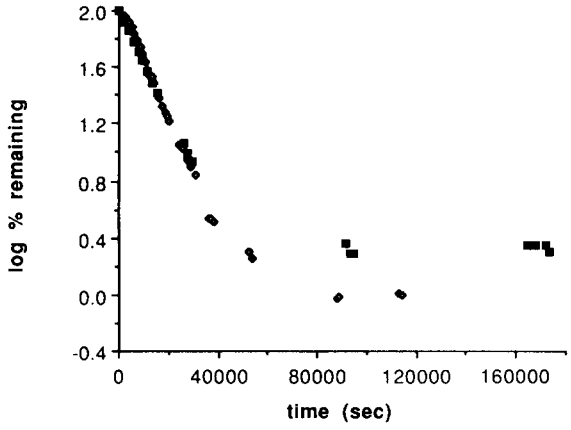


Fig. 1. Changes in log% remaining of rifampicin (calculated from molar concentrations) as a function of time for initial rifampicin concentrations of 10.5 μg/ml (◇) and 21.8 μg/ml (■) at 37°C in 0.01 M hydrochloric acid.

viously (Gallo and Radaelli, 1976). In the present study, the presence of **II** in reaction mixtures was suggested by co-elution with an authentic specimen (Merrell-Dow MDL 29,038), although isolation of reaction products was not attempted. Under the acidic conditions of this study, and in the presence of ascorbic acid (Ratti et al., 1981), no other degradation products were observed. Semilog plots of [**I** + **II**] vs time were linear for the reaction duration. Ascorbic acid did not interfere with the HPLC analyses. In addition, rate constants did not change when a 2-fold change in ascorbic acid concentration was used at pH 1. No studies were performed to examine possible catalytic effects of the buffering agents.

As has been previously observed for nitrofurantoin and ebifuramin, hydrolysis of rifampicin was not expected to follow pseudo first-order kinetics. Semilog plots of [**I**] as a function of time appeared to be biexponential, with the initial phase appearing to be linear for about three half-lives, before curvature to a slower linear terminal phase (Figs 1–3). In addition, the slope of the terminal phase was parallel to that of the decline in concentration of **II** (Fig. 2), indicating that **I** and **II** are in equilibrium.

Treatment of concentration-time data

The experimental data was analyzed by two methods:

(i) Pseudo-first order kinetics were assumed, based on the linearity seen for about three half-lives. The rate constants calculated in this way are referred to as apparent first-order initial rate constants (k_{init}).

(ii) Reversible kinetics involving a pseudo-second order reverse reaction (Moore and Pearson, 1981) as previously used to describe the hydrolysis of ebifuramin (Prankerd and Stella, 1989). The forward rate constants calculated by this method are called apparent first-order forward rate constants (k_f).

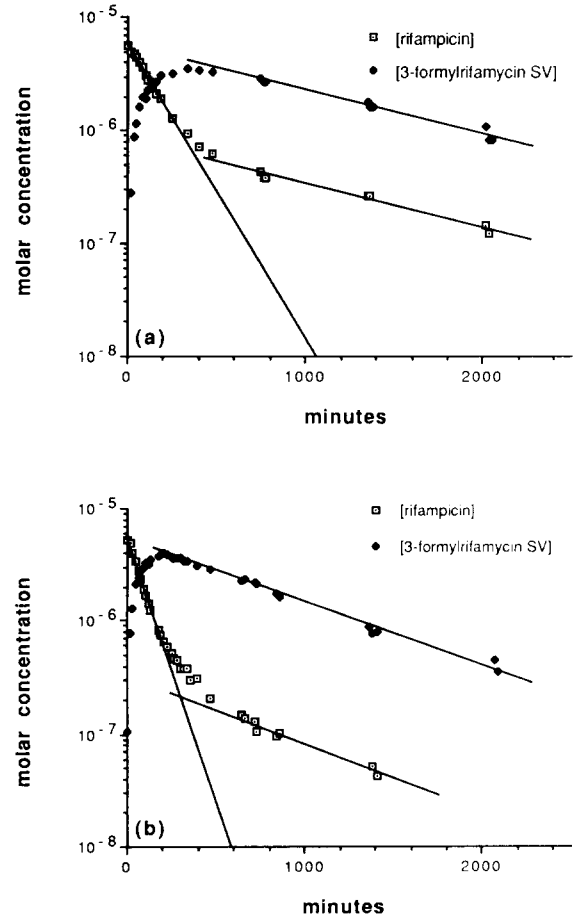


Fig. 2. Time-dependent changes in concentration of rifampicin and 3-formylrifamycin SV at pH 2.8 (37°C, $I = 0.1$ M) in the absence (a) and presence (b) of 1.0 M formaldehyde. For (a), the apparent initial first-order rate constant is 9.96×10^{-5} s^{-1}, and for (b), 1.80×10^{-4} s^{-1}.

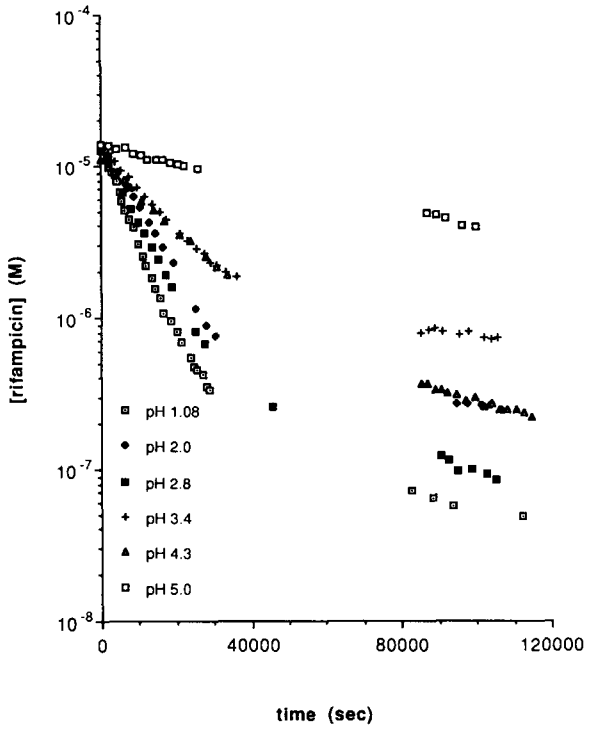


Fig. 3. Representative plots of time-dependent changes in concentration of rifampicin at various pH values (37°C, $I = 0.1$ M) and an initial rifampicin concentration of 21 μg/ml. Data from longer intervals showing all of the terminal phase of the reaction have been omitted for clarity.

The rate constants are reported in Tables 1 and 2. The second method can also be used to calculate the apparent second-order reverse rate constant and the equilibrium constant for each reaction (Eqn 2). These have not been reported as they were found to be too imprecise. This was due to the difficulty in accurately determining the molar extent of reaction at equilibrium, which was very close to completion of the reaction. This is indicated by the percent remaining of **I** at equilibrium after correction for the terminal phase reaction (Tables 1 and 2).

TABLE 1

Apparent first-order initial rate constants (k_{init}), apparent first-order forward rate constants (k_f) and corrected % remaining for hydrolysis of I in 0.01 M hydrochloric acid at 37°C, with two different initial rifampicin concentrations

Initial conc (μg/ml)	k_{init} ($\times 10^5$) (s^{-1})	k_f ($\times 10^5$) (s^{-1})	% **I** at t_0 (corrected)
1.05	9.74 ± 0.22 [a]	9.21 ± 0.35	2.14 ± 0.05
2.18	8.31 ± 0.05	8.90 ± 0.04	3.00 ± 0.06

[a] Errors are calculated from the range of replicate measurements.

TABLE 2

Apparent initial (k_{init}) and forward (k_f) rate constants for hydrolysis of I in buffers ($I = 0.1$ M) at 37°C as a function of pH

pH	k_{init} ($\times 10^5$) (s^{-1})	k_f ($\times 10^5$) (s^{-1})	% **I** at equilibrium (corrected) [a]
1.08	14.6 ± 0.25 [b]	15.3 ± 0.9	4.50
2.02	8.31 ± 0.03	8.90 ± 0.02	3.00
2.82	10.97 ± 0.25	10.92 ± 0.10	0.89
3.41	6.19 ± 0.08	5.62 ± 0.17	5.23
4.36	5.29 ± 0.07	4.98 ± 0.10	2.75
5.0	1.21 ± 0.03	1.23 ± 0.10	7.51

[a] Calculated from the molar extent of reaction at equilibrium (x_e) and the initial [**I**].
[b] Errors are calculated from the range of replicate measurements.

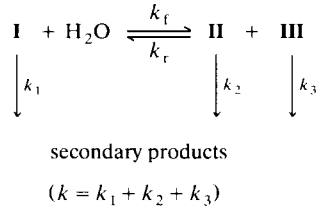
Concentration-dependent hydrolysis of rifampicin in 0.1 M hydrochloric acid

The concentration-dependent hydrolysis of rifampicin was studied in 0.01 M hydrochloric acid at two different initial concentrations of **I**. Fig. 1 shows the change in concentration of **I** with time for these solutions. As in the previous study on ebifuramin hydrolysis (Prankerd and Stella, 1989), the plots of rifampicin loss as a function of time (Fig. 1) are interpreted in terms of a fast reversible reaction leading to a dynamic equilibrium, followed by one or more parallel slower reactions which obey apparent pseudo-first order kinetics. The apparent first-order initial (k_{init}) and forward rate constants (k_f) are reported in Table 1. The slower reaction(s) corresponds to loss of either rifampicin or its degradation products by parallel pseudo-first order reactions (see Fig. 2). As would be expected for a reversible reaction (Scheme 1), the initial phase of the curves in Fig. 1 indicates that the equilibrium dissociation of rifampicin to **II** and **III** is more complete

as the initial concentration of rifampicin is decreased. Concentration-dependent degradation of rifampicin was previously observed by Seydel (1970), but mistakenly attributed to precipitation of **II**.

Trapping experiment

The use of formaldehyde as a trapping agent has been described previously for hydrolyses of ebifuramin (Prankerd and Stella, 1989). The added aldehyde (in high concentration) competes with the liberated aldehyde (**II**, in the case of rifampicin) for the amine reaction product (**III**, in the present study). For ebifuramin, addition of 0.6 M formaldehyde to the reaction solutions (pH 1–5) converted the biphasic log concentration-time plots to monophasic apparent first-order plots with rate constants similar to k_f at each pH. The results of the trapping experiment for rifampicin (chloroacetate buffer, pH 2.8) are compared to a similar reaction solution without formaldehyde in Fig. 2. The figure shows that the presence of 1.0 M formaldehyde increased the initial slope of the log concentration vs time plot. However, the plot is still biphasic (due to the competing reverse reaction). Thus, the concentration of formaldehyde was not sufficient to convert the reversible hydrolysis reaction to an irreversible reaction. This result is interesting, by comparison with the complete conversion to apparent first-order behavior seen for ebifuramin (Prankerd and Stella, 1989), and indicates a very high affinity of the 1,4,8-trihydroxynaphthalene-3-carboxaldehyde group of 3-formylrifamycin SV for the liberated amine (1-amino-4-methylpiperazine). It was decided not to pursue this approach to even higher concentrations, as other effects of the added formaldehyde (such as polarity) were expected to be large and rate constants would not be expected to be close to k_f for the reversible reaction. In the study of ebifuramin (Prankerd and Stella, 1989), the apparent first-order rate constants in the presence of the trapping agent were typically 15–25% higher than those obtained using Eqns 1 and 2. These differences were ascribed to medium effects (presumably on the transition state) from the added high concentration of formaldehyde.



Scheme 2.

Hydrolysis of rifampicin as a function of pH

The hydrolysis of rifampicin was studied in the pH range 1–5 and at an initial concentration of approx. 21 μg/ml. Plots of the time-dependent concentrations of **I** (Fig. 3) at pH values in the range of 1–5 did not follow pseudo-first order kinetics for more than about three half-lives, very similar to those in Fig. 2. Fitting the data up to three half-lives to the standard equation for pseudo-first order kinetics gave the apparent initial rate constants (k_{init}) in Table 2. The concentration-time data were also analyzed as described above for reversible kinetics with a second order reverse reaction (method (ii) above). As previously described (Prankerd and Stella, 1989), a feathering method was used to correct the concentration-time plots for the secondary degradation, described by Scheme 2, using Eqn 3:

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

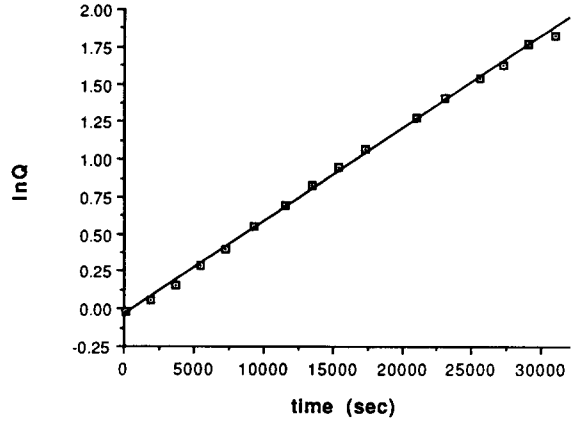


Fig. 4. Typical plot of $\ln[ax_e + x(a - x_e)]/a(x_e - x)$ (= ln Q) as a function of time, according to Eqn 1.

where k is the observed slope of the linear terminal phase of the plot of $\ln[\mathbf{I}]_t$ vs time, t, at each pH value. The corrected concentrations $[\mathbf{I}]_t$ were then employed in Eqn 1. The rate constants from the terminal slopes (k) are regarded as apparent first-order rate constants which describe the overall loss of rifampicin after a pseudo-equilibrium had been reached at each pH value. It was assumed that the rate constants for the secondary degradation reactions (k_1, k_2, k_3) were all pseudo-first order for the reaction conditions. The apparent first-order forward rate constants obtained from Eqn 1 are reported in Table 2. In all cases, the plots of the left-hand side of Eqn 1 vs time had zero intercept and were linear with standard errors of less than 10% of the slope at worst. A typical plot is shown in Fig. 4. The precision of fitting the data to Eqn 1 was less than previously observed for ebifuramin. This was largely due to the difficulty in accurately determining the molar extent of reaction at equilibrium. The percent remaining of rifampicin, corrected according to Eqn 3, was very small when equilibrium was reached (Table 2). For this reason, the values for k_r (0.05–1.0 M^{-1} s^{-1}) and K_{eq} (5×10^{-4}–1×10^{-3} M) were very imprecise and no relationship with pH could be recognized. The values for k_f were found to be similar to those for k_{init} and their pH-rate profiles are shown in Fig. 5. The apparent rate constants (k_{init} and k_f) range from 1.5×10^{-4} to 1.2×10^{-5} s^{-1} for pH 1–5. The hydrolysis of ebifuramin is rather slower, as the rate constants (k_f) varied from 2.8×10^{-5} to 6.9×10^{-7} s^{-1} for the same pH range and temperature.

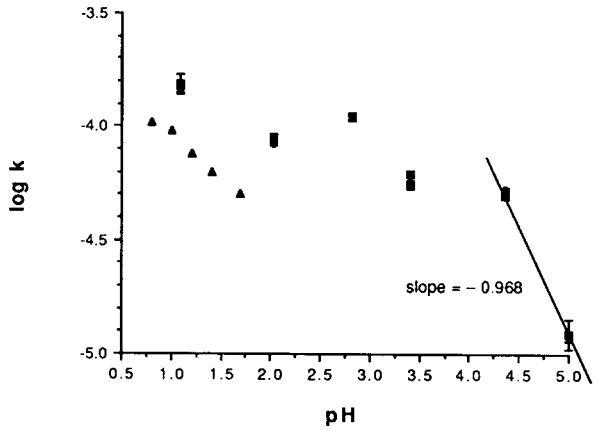


Fig. 5. Dependence of log k_{init} (◇) and log k_f (■) on pH for the hydrolysis of rifampicin (37°C, $I = 0.1$ M) and an initial rifampicin concentration of 21 μg/ml. Error bars are from Table 2. The data from Seydel (1970) is also plotted (▲).

pH-rate profiles

The pK_a values for rifampicin have been reported previously (Maggi et al., 1966; Gallo and Radaelli, 1976; Kenny and Strates, 1981). Values reported were 1.7 (from spectral data, assigned to the C_1, C_4 and C_8 phenolic hydroxyl groups) and 7.9 (potentiometric titration with sodium hydroxide, assigned to one of the piperazine nitrogens). However, these assignments should be questioned. In particular, the value of $pK_a = 1.7$ seems to be too low for the aromatic hydroxyl system. An alternative interpretation would assign the spectrophotometric value of 1.7 to the azomethine nitrogen, consistent with other azomethines, e.g. diazepam, $pK_a = 3.3$ (Barrett et al., 1973) and ebifuramin, $pK_a < 2$ (Prankerd and Stella, 1989). The value of 7.9 should then be assigned to ionization of the C_1, C_4 and C_8 aromatic hydroxyl system. Also, the piperazine N-CH_3 might be expected to exhibit an ionization step with a pK_a value in the range 6–8. For example, *N*-nitroso-*N'*-methylpiperazine, which is similar to the *N*-methylpiperazine side-chain moiety of **I**, has pK_a 5.93 (Perrin, 1965). This ionization would not be detected by the procedures reported so far (Maggi et al., 1966) for rifampicin.

The pH-rate profiles for k_f and k_{init} (Fig. 5) are not easily interpreted. Similar difficulties in interpretation of the pH-rate profiles for hydrolysis of ebifuramin were previously reported (Prankerd and Stella, 1989), and a partial pH-rate profile for reversible hydrolysis of the diazepam azomethine bond is also irregular (Han et al., 1977). The increased rate constants for hydrolysis of rifampicin at pH 2.8, compared to those at pH 2.0 and 3.4 seem to be real. There is a similar discontinuity at about the same pH value in the corresponding pH-rate profile for diazepam (Han et al., 1977). This discontinuity was ascribed to facile reformation of diazepam from its acyclic

hydrolysis product. Differences are seen on comparing Fig. 5 with the corresponding pH-rate profile for ebifuramin, where there is a more-or-less continuous decrease in log k_f as pH is raised. Below pH 2, the profile for ebifuramin has a slope which approaches negative unity. For rifampicin, a unit negative slope is seen between pH 4.3 and 5.0, but not at lower pH values. A similar unit negative slope is seen for diazepam hydrolysis above its pK_a (Han et al., 1977). These negative slopes may result either from specific acid catalysis, or from a kinetic equivalent involving acid-base equilibria of the substrate or a transient carbinolamine intermediate. Interpretation of these pH-rate profiles is difficult without a knowledge of the pK_a for the transient carbinolamine presumed to be formed on hydration of the azomethine bond.

Kinetics for hydrolysis of the azomethine bond of **I** have previously been reported in a systematic fashion only over a very narrow pH range (0.75–1.75) (Seydel, 1970). The pH-rate profile given in Fig. 5 is in qualitative agreement with that of Seydel. The present data shows that at 37°C, the rate of azomethine bond hydrolysis decreases by about 0.5 log unit over the pH range 1–4.3, and more rapidly above this pH. It is therefore likely that azomethine cleavage continues after the drug leaves the more acidic milieu of the stomach (pH 1–3.5). Over this pH range, the half-life of rifampicin ranges from about 1.2 to 3.1 h at 37°C.

Conclusions

Rifampicin in the presence of an antioxidant appeared to undergo fast reversible hydrolytic cleavage at its azomethine bond followed by slower secondary reaction(s). Because of the reversible nature of the initial reaction step, the overall loss of rifampicin from solution was dependent on initial concentration. As the studies were performed at low initial drug concentration, the second-order reverse reaction appeared to contribute to only a very limited extent, and the rate of loss approached pseudo-first order behavior. It is apparent from this study and those on diazepam (Han et al., 1977) and ebifuramin (Prankerd and Stella, 1989) that a pattern is emerging of unusual pH-rate profiles for hydrolysis of azomethine compounds which requires additional basic study to fully comprehend.

Acknowledgements

Financial support from SmithKline Beecham is gratefully acknowledged. The authors would like to express their thanks to Ciba-Geigy and to Merrell-Dow for generous gifts of rifampicin and authentic samples of its degradation products.

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