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Acid-catalyzed hydrolysis of (R)-4-[(1-methyl-3-oxo-1-butenyl)amino]-3-isoxazolidinone, a prodrug of cycloserine

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Summary

Hydrolysis of (R)-4-[(1-methyl-3-oxo-butenyl)amino]-3-isoxazolidinone (I), a prodrug of D-cycloserine, to (R)-4-amino-3-isoxazolidinone (D-cycloserine) and 2,4-pentandione is general acid-catalyzed by carboxylic acids. Rectangular hyperbolic plots of pseudo first-order rate constants vs total carboxylic acid buffer concentrations suggest a change in the rate-determining step, from protonation of I at low acid concentrations to hydrolysis of protonated I at high carboxylic acid concentrations.

Introduction

A fixed ratio combination of (R)-4-[(1-methyl-3-oxo-1-butenyl)amino]-3-isoxazolidinone (I) and 2-deuterio-3-fluoro-D-alanine (II), with good antibacterial activity and low toxicity (Kahan and Kropp, 1975; Kropp et al., 1975; Kollonitsch et al., 1975; Kahan et al., 1975), has been entered into clinical trial (Shen, 1983). At therapeutic doses, II prevents bacteria from synthesizing D-alanine for cell wall synthesis but at higher doses II fosters reproduction of bacteria by substituting for

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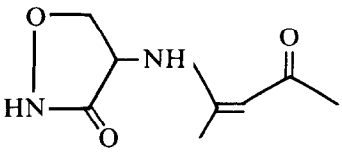
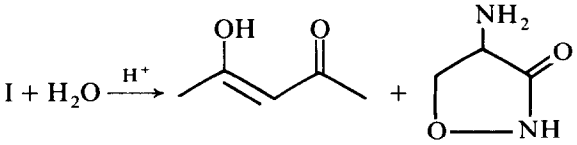


I

its natural isotere, D-alanine. The role of I is to prevent this by releasing in vivo D-cycloserine, which inhibits D-alanyl-D-alanine synthetase, the enzyme that converts II to fluoro-D-alanyl fluoro-D-alanine for cell wall synthesis.

Prodrug I was developed as a stabilized form of D-cycloserine, which undergoes dimerization in aqueous media and in the solid state (Jensen et al., 1980; Lassen and Stammer, 1968). The release of D-cycloserine from I was reported to be 'a complex function of several factors...' (Jensen et al., 1980).

Here we report the kinetics and mechanism of the acid-catalyzed hydrolysis of I (Eqn. 1).



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Experimental

Apparatus

A Gilford Model 2400 spectrophotometer, a Cary Model 118C spectrophotometer, a Tamson T9 bath, and a Radiometer PHM 26 were used. A Hewlett-Packard 9820A calculator and the Cyber 173 computer (S.U.N.Y., Buffalo) were used to do linear and non-linear regression using least-squares and the BMDPAR non-linear regression programs.

Reagents and compounds

Certified ACS grade inorganic reagents and organic solvents were purchased from Fisher Scientific. D-Cycloserine, 2,4-pentadione, D_2O, and DCl were purchased from Aldrich Chemicals. (R)-4-[(1-methyl-3-oxo-1-butenyl)amino]-3-isoxazolidinone (I) was synthesized by the method of Jensen et al., (1980), m.p. 146°C (Lit. 146°C) and had IR, UV and NMR spectral properties identical to those reported.

Kinetics

Reactions were carried out in H_2O at 30 ± 0.1°C. The calculated ionic strength was adjusted with KCl. The pH of each solution was determined before and after runs and the pH remained constant during runs. Cuvettes (3 ml) were charged with dilute HCl (DCl) or carboxylate buffer and allowed to reach 30°C. Reactions were started by adding I in CH_3OH via a micropipet to the cuvette to give a final

concentration of ca. 2×10^{-5} M I. Reactions were monitored by following the decrease in absorbance at 311 nm and were carried out under pseudo first-order conditions. Pseudo first-order rate constants, k_{obsd}, were obtained from slopes of plots of ln $((OD_i - OD_\infty)/(OD_t - OD_\infty))$ vs time. Plots were linear to at least 90% reaction. The UV spectra of reactions at completion exactly resembled those of equimolar mixtures of D-cycloserine and 2,4-pentandione. The pK_a of I, 3-hydroxy-isoxazoline'tautomer, was obtained by dissolving I in excess standard KOH and back-titrating with standard HCl. Ionization constants calculated at several pH values were averaged to give $pK_1 = 5.66 \pm 0.05$. pD was calculated from pH by adding 0.4 to it (Fife and Bruice, 1961).

Results

Hydrolysis of I in the pH (pD) range 0.7–7.12 (0.47–6.5) follows the rate law of Eqn. 2 where a_H (a_D) is the activity of hydrogen (deuterium) ion and A, B, C and D are constants (Table 1) obtained by fitting the experimental data to Eqn. 2

$$-d[I]/dt[I] = k_{obsd} = (Aa_H + Ba_H^2)/(C + a_H + Da_H^2) \quad (2)$$

using the BMDPAR non-linear regression program. This equation, suggested by the mechanism of Scheme I (Discussion section), was one of several tried and it gave the best fit. Fig. 1 shows the pH–rate profile that was obtained for hydrolysis of I in water.

Hydrolysis is catalyzed by carboxylate buffer solutions. In the concentration range 0–0.2 M methoxyacetic acid, k_{obsd} is practically linearly dependent on total buffer concentration with $(\text{slope}/f_{HA}) = 6$ (pH 2.8) and 5.3 (pH 3.43) $M^{-1} \cdot min^{-1}$. Here, f_{HA} is the mole fraction of carboxylic acid in the buffer.

Over a larger concentration range of buffer, plots of k_{obsd} vs total concentration of buffer begin to show a saturation effect (Fig. 2) and provide evidence for more complex kinetics. For example, at pH 2.8, $1/k^B_{obsd} = 0.3/[CH_3OCH_2CO_2H]_{total} + 0.313$ ($r = 0.9999$) and at pH 2.72, $1/k^B_{obsd} = 0.19/[ClCH_2CO_2H]_{total} + 0.145$ ($r = 0.9999$). Here, k^B_{obsd} is the pseudo first-order rate constant for buffer catalysis and is equal to the experimental pseudo first-order rate constant less k_{obsd} for H_3O^+ catalysis.

For hydrolysis catalyzed by acetate buffer, pH 3.65–4.65, curvature in plots of k_{obsd} vs $[\text{buffer}]_{total}$ (Fig. 2) is even more apparent and the rate law of Eqn. 3 is

TABLE 1

VALUES OF CONSTANTS OF EQN. 2 FOR HYDROLYSIS OF I IN L_2O

Solvent	A (min^{-1})	B ($M^{-1} \cdot min^{-1}$)	C (M)	D (M^{-1})
H_2O	0.204	157	2.5×10^{-6}	32.9
D_2O	0.200	180	1.2×10^{-6}	81.1

followed. Table 2 gives the pH-dependent values of slopes and

$$k^{B}_{obsd} = M[HA]_{total}/(N + [HA]_{total}) \quad (3)$$

intercepts obtained from plots of $1/kB_{obsd}$ vs $1/[HA]_{total}$.

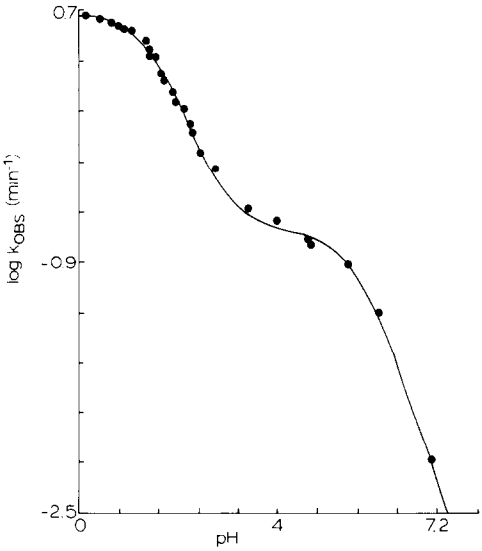


Fig. 1. Plot of the logarithm of the pseudo-first-order rate constant, k_{obsd}, vs pH for hydrolysis of I catalyzed by hydronium ion.

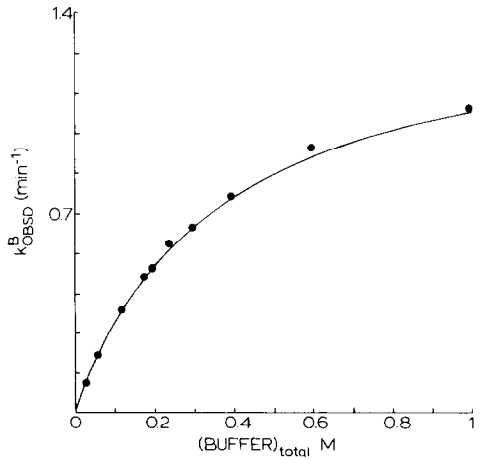


Fig. 2. Plot of the pseudo-first-order rate constant for acetic acid–acetate buffer catalysis, k^{B}_{obsd}, only vs the molar concentration of acetic acid plus acetate ion for hydrolysis of I, pH = 3. $\mu = 0.5$ (KCl), $t = 30 \pm 0.1$°C.

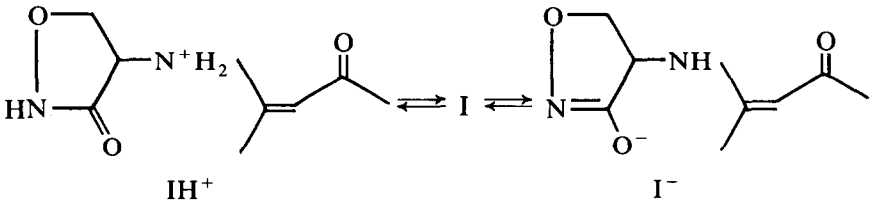
TABLE 2

VALUES OF SLOPES AND INTERCEPTS FROM PLOTS OF $1/k^{B}_{obsd}$ vs $1/[HA]_{total}$ ACCORDING TO EQN. 3 FOR HYDROLYSIS OF I IN ACETATE BUFFERS

pH (pD)	Slope N/M (M·min)	Intercept 1/M (min)	r
3.65	0.273	0.391	0.9999
3.76	0.312	0.502	0.9981
3.92	0.265	0.604	0.9997
4.00	0.305	1.115	0.9872
4.65	0.446	3.135	0.9988
(5.08)	0.751	2.614	0.9993

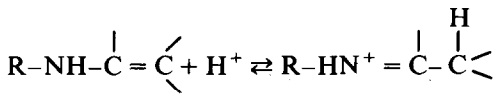
Discussion

In the pH range of this study, 3 species exist. At higher pHs, I ($pK_1 = 5.66$) is in equilibrium with 3-hydroxyisoxazoline anion I^-. At lower pHs, I is in equilibrium with N-protonated enamine IH^+. Of these, I and I^- predictably



IH^+ I I^-

should react with acids to form hydrolytically labile immonium ions (Eqn. 4); IH^+ should not. These predictions are based on the results of Stamhuis and Maas



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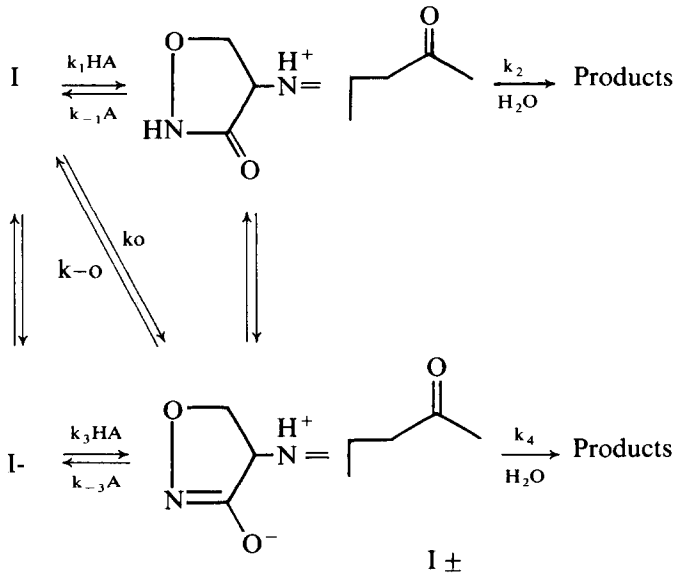
(1965), Maas et al. (1967), Coward and Bruice (1969) and Sollenberger and Martin (1970), who studied the hydrolysis of simpler enamines.

A minimal mechanism for hydrolysis of I to D-cycloserine and pentan-2,4-dione is shown in Scheme 1. Here k_0 is the rate constant for spontaneous reaction of I to give zwitterionic imine $I^{\pm}$. This process could involve an intramolecular proton transfer.

Buffer carboxylic acid catalysis implicates rate-determining protonation of carbon atom 3 (Stamhuis and Maas, 1965; Guthrie and Jordan, 1972) and for hydrolysis of I catalyzed by the hydronium ion, we assumed that the k_1, k_0 and k_3 steps (Scheme 1) are rate determining. The rate law of Eqn. 5 can be derived by:

$$\text{rate}/[I]_{total} = k_{obsd} = \left((k_0 + k_3K_1)a_H + k_1a_H^2\right)/\left(K_1 + a_H + a_H^2/K_{IH^+}\right) \qquad (5)$$



Scheme 1

using the total concentration of I species, $[I]+[I^-]+[IH^+]$, and the equilibrium constants $K_1 = [I^-][H^+]/[I]$ and $K_{IH^+} = [I][H^+]/[IH^+]$. Eqn. 5 has the form of Eqn. 2 derived from experiments. From the data of Table 1, $(k_0 + k_3k_1) = 0.204\ min^{-1}$, $k_1 = 157\ M^{-1} \cdot min^{-1}$, $K_1 = 2.5 \times 10^{-6}$ M, and $K_{IH^+} = 3.04 \times 10^{-2}$ M. The value of K_{IH^+} is in the range of values found via kinetics for β-anilinocrotononitriles and β-aminocinnamonitriles (Coward and Bruice, 1969). The value of K_1 is in good agreement with the value 2.2×10^{-6} M obtained by tritration (Experimental section).

Carboxylic acid buffer catalysis provides evidence of proton transfer in the critical transition state of this multistep reaction and for a change of mechanism, dependent on buffer concentration (Fig. 2).

In acetate buffers, pH 3.65–4.65, the major prodrug species is I, and approximately, Scheme 1 may be simplified to Scheme 2 for buffer catalysis.

$$I \underset{k_{-1}A}{\overset{k_1HA}{\rightleftarrows}} I^+ \xrightarrow[H_2O]{k_2} \text{Products}$$

Scheme 2

For this mechanism, the rate law of Eqn. 6 can be written by assuming a steady-state condition for I^+. Here f_{HA} and f_A are the mole fractions of

$$\text{rate}/[I] = k^B_{obsd} = k_1k_2f_{HA}[HA]_{total}/(k_{-1}f_A[HA]_{total} + k_2) \qquad (6)$$

acetic acid and acetate ion in the buffer. A comparison of Eqns. 3 and 6 shows that

the intercepts of Table 2 are equal to $(k_{-1}K_{HA}/k_1k_2a_H)$ and the slopes are equal to $(1/k_1f_{HA})$. Here K_{HA}, the dissociation constant of acetic acid, has the value 1.78×10^{-5} M (Albert and Serjeant, 1962).

The data of Table 2 may be used to calculate $k_1 = 3.91\ M^{-1} \cdot min^{-1}$ and $(k_{-1}/k_2) = 24.5\ M^{-1}$ for acetic acid catalysis. In a similar way, k_1s and partitioning ratios (k_{-1}/k_2)s were calculated for chloracetic acid and methoxyacetic acid catalysis from the data in the Results section. All buffer catalysis constants, including those for reactions of I with acetic acid in deuterium oxide, are collected in Table 3.

The buffer concentration-dependent mechanism change suggested by Fig. 2 is explained by Scheme 2 and the limiting forms of Eqn. 6. When carboxylic acid-carboxylate ion buffer concentration is low, protonation of I is rate determining. When buffer concentration is high, reaction of immonium ion I^+ with water to form carbinolamine is rate determining. This interpretation was suggested by Guthrie and Jordan (1972) to explain similar results with ethyl β-cyanomethylaminocrotonate.

Data (Table 3) for reactions of I catalyzed by hydronium ion and carboxylic acids are related by the Bronsted relationships, $\log k_1 = (-0.25 \pm 0.03)\ pK_a + 1.7 \pm 0.1$ and $\log (k_{-1}/k_2) = (0.58 \pm 0.09)\ pK_a - 1.32 \pm 0.3$. Because k_2s are the same for each buffer, the β value 0.58 is for deprotonation of I^+ via k_{-1}.

Classically interpreted, $\alpha = 0.25$ signifies an early transition state as I is protonated to form I^+. For protonation of enamines, αs vary from 0.3 for 1-N-morpholino-1-isobutene (Stamhuis and Maas, 1965) to 0.6 for β-anilinocinnamonitrile (Coward and Bruice, 1969) and 0.5 for propiophenone morpholine enamine (Sollenberger and Martin, 1970). These values, for diversely structured enamines, are roughly in accord with the effect of substituents on the electron density on the carbon atom that is protonated and with the basicity of the nitrogen atom.

Solvent deuterium isotope effects support the mechanism of Schemes 1 and 2. For reactions of I in acetic acid-acetate buffers, $k_1(H_2O)/k_1\ (D_2O) = 1.8$ (Table 3). This less-than-maximum value for an isotope effect for a proton transfer to carbon is in accord with an early transition state, as suggested by $\alpha = 0.25$, and with a rate-determining proton transfer. The isotope effect on the reverse deprotonation of I^+ is 2.8 (Table 3).

TABLE 3

RATE CONSTANTS AND PARTITIONING RATIOS FOR REACTIONS OF I IN AQUEOUS ACID

Acid	pK_a	$k_1\ (M^{-1} \cdot min^{-1})$	$k_{-1}/k_2\ (M^{-1})$
H_3O^+	−1.74	157	
$ClCH_2CO_2H$	2.85 [a]	9.23	1.81
$CH_3OCH_2CO_2H$	3.53 [a]	3.96	6.64
CH_3CO_2H	4.76 [a]	3.91	24.5
CH_3CO_2D	5.26 [b]	2.21	8.8

[a] Albert and Serjeant (1962).

[b] Bunton and Shiner, Jr. (1961).

For reactions of I with carboxylic acids, (k_{-1}/k_2) partitioning ratios increase as pK_as of carboxylic acids increase (Table 3). That is, partitioning of I^+ favors I increasingly as the basicity of the carboxylate anions increases. This is exactly what would be predicted for deprotonation of I^+.

For deprotonation of I^+ via k_{-1}, $\beta = 0.58$. As required, the transition state for the reverse deprotonation of I^+ is late. (The transition state for protonation of I is early.) Theory requires that $(\alpha + \beta) = 1$. The experimental result (0.84 ± 0.1) is acceptably close.

We conclude that I hydrolyzes via the immonium ion I^+ ($I^{\pm}$) which paritions in a kinetically detectable way in acetate buffers between I and products. The pH–rate profile (Fig. 1) shows that the stability of I is greatest at high pH. Eqn. 2 predicts that 50% degradation of I would occur in ca. 2.9 h at pH 7.3 at 30°C and that I would rapidly decompose in gastric juice to D-cycloserine. Our failure to detect appreciable hydrolysis of I at pH 10 during 72 h (the estimated k_{obsd} was that predicted from eqn. 2) suggests that the alternative Michael reaction of I with hydroxide ion is disfavored on steric and electrostatic grounds.

It remains to be established whether pentan-2,4-dione will prove useful as a reagent for prodrug synthesis. Predictably, derivatives formed from alcohol-containing drugs would be acid labile and base labile (Fedor et al., 1973; Chu et al., 1976).

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