The Interconversion Kinetics, Equilibrium, and Solubilities of the Lactone and Hydroxyacid Forms of the HMG-CoA Reductase Inhibitor, CI-981

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The pH dependence of the interconversion kinetics, equilibrium, and solubilities of the lactone and hydroxyacid forms of the HMG-CoA reductase inhibitor, CI-981 ([*R*-(*R**,*R**)]-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-hepatonic acid), are important considerations when chosing and developing one of the forms of these compounds. Over a pH range of 2.1 to 6.0 and at 30°C, the apparent solubility of the sodium salt of CI-981 (i.e., the hydroxyacid form) increases about 60-fold, from 20.4 μg/mL to 1.23 mg/mL, and the profile yields a pK_a for the terminal carboxyl group of 4.46. In contrast, over a pH range of 2.3 to 7.7 and also at 30°C, the apparent solubility of the lactone form of CI-981 varies little, and the mean solubility is 1.34 (±0.53) μg/mL. The kinetics of interconversion and the equilibrium between the hydroxyacid and the lactone forms have been studied as a function of pH, buffer concentration, and temperature at a fixed ionic strength (0.5 *M*) using a stability-indicating HPLC assay. The acid-catalyzed reaction is reversible, whereas the base-catalyzed reaction can be treated as an irreversible reaction. More specifically, at pH <6, an equilibrium favoring the hydroxyacid form is established, whereas at pH >6, the equilibrium reaction is no longer detectable and greatly favors the hydroxyacid form. The rate constant for lactone formation, k_1, is well described by specific acid-catalyzed and spontaneous lactonization pathways, whereas the rate constant for lactone hydrolysis (or hydroxyacid formation), k_2, is well described by specific acid-, water-, and specific base-catalyzed pathways.

KEY WORDS: CI-981; HMG-CoA reductase inhibitor; stability; solubility.

INTRODUCTION

HMG-CoA (3-hydroxy-3-methyl-glutaryl-coenzyme A) reductase inhibitors are used for the treatment of hypercholesterolemia since the inhibition of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis, lowers plasma concentrations of low-density lipoprotein and total cholesterol (1,2). The currently marketed inhibitors are administered either as the sodium salt of the pharmacologically active hydroxyacid form (i.e., pravastatin) or as the corresponding lactone form (i.e., lovastatin and simvastatin). The lactone has been considered a prodrug which converts to the active hydroxyacid *in vivo*.

The focus of this study is the pH dependence of the interconversion kinetics, equilibrium, and solubilities of the lactone and hydroxyacid forms of CI-981, [*R*-(*R**,*R*)]-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-hepatonic acid (Fig. 1). CI-981 is a potent HMG-CoA reductase inhibitor that is currently in Phase 2 clinical trials (3,4). The physicochemical properties of the lactone and hydroxyacid forms of HMG-CoA reductase inhibitors impact their formulation and biologic performance, hence they are important considerations when chosing and developing one of these forms.

MATERIALS AND METHODS

Materials

CI-981 and the corresponding lactone form were synthesized by the Chemistry Department at Parke-Davis Pharmaceutical Research (Ann Arbor, MI). Synthetic procedures have been reported previously (4,5). All other chemicals were of reagent or analytical grade, and the water was distilled and deionized.

Analytical Methods

The pH measurements were performed at the temperatures of the studies with an Accumet pH meter 925 and a Ross combination glass electrode, using a two-point calibration with certified buffer solutions. The stability-indicating HPLC analyses were performed on an HP 1090 liquid chromatograph equipped with a diode-array detector operating at a fixed wavelength of 246 nm. The column was a Beckman Ultrasphere ODS (4.6 mm × 25 cm) 5-μm column. The mobile phase was composed of a 60:40 mixture of acetonitrile: 50 m*M* sodium acetate in water, where the pH was adjusted to 4.0 with glacial acetic acid. The injection volume was 20 μL, and the eluent flow rate was 1.0 mL/min. The hydroxyacid form had a retention time (t_R) of about 4 min, whereas the lactone form had a t_R of about 7 min.

Solubility Studies

The aqueous solubilities of the sodium salt of the hydroxyacid and the lactone forms of CI-981 were determined as a function of pH at 30°C. (Ionic strength was not adjusted.) Excess solid sodium salt (~20 mg) or lactone (~10 mg) was placed in 10-mL screw-cap vials containing 10.0 mL of water. These mixtures were adjusted to the desired pH using either concentrated HCl or NaOH. The capped vials were placed on a Van Kel rotating-bottle apparatus which was placed in a 30°C water bath and rotated at 50 rpm. One-milliliter samples of each suspension were removed from the vials at various times and placed in 1.5-mL polypropylene micro test tubes. These tubes were then spun in a centrifuge for 40 min at 14,000 rpm. The resulting supernatant was assayed by HPLC, and the apparent solubilities were obtained by interpolation from an appropriate standard curve. The pH of the supernatant was also determined. Samples were taken over a 17-hr interval for the sodium salt and over

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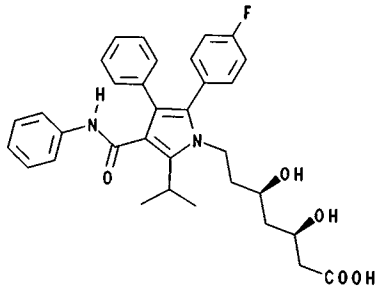


Fig. 1. The structure of CI-981, [*R*-(*R**,*R**)]-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid.

a 48-hr interval for the lactone; longer equilibration times were not used in order to minimize conversion to the alternate chemical form.

Stability Studies

The interconversion kinetics and the associated equilibrium were determined, with dilute aqueous solutions of the sodium salt of the hydroxyacid or lactone forms of CI-981, as a function of pH and temperature at a constant ionic strength of 0.5 *M* (with NaCl). The pH of the solutions was maintained with either HCl or formate, acetate, phosphate, or borate buffers; and the pH studied ranged from 1.3 to 9.0. When buffers were used, the kinetics were measured as a function of buffer concentration (i.e., 25, 50, 75, and 100 m*M*) at a constant pH, and if buffer catalysis was observed, the reported rate constants were those extrapolated to zero buffer concentration.

The kinetic studies were initiated by adding 100 μL of a methanolic stock solution (~1 mg/mL) of the hydroxy acid or lactone forms to 10-mL volumetric flasks of the reaction mixtures which were temperature equilibrated in a circulating water bath. At appropriate time intervals, samples were withdrawn, quenched in an ice-water bath, and assayed for both the hydroxyacid and the lactone forms. At the completion of each kinetic run, the pH of the reaction mixtures were measured at the temperature of the study.

In HCl and in the formate and acetate buffers, the hydroxyacid form was used as the starting material, and its conversion to the lactone was followed to equilibrium. The pseudo-first-order rate constant for lactone hydrolysis or hydroxy acid formation, k_2, and the associated equilibrium constant, K_{eq} (which is the ratio of the lactone to the hydroxyacid form of CI-981 at equilibrium), were obtained by fitting [using PCNONLIN (SCI, Lexington, KY) and Nelder-Mead simplex algorithm] the lactone formation data to the following equation:

$$[\text{Lactone}] = \frac{K_{eq} \cdot [\text{CI-981}]_o}{(K_{eq} + 1)} [1 - \exp[-(k_2 + K_{eq} \cdot k_2)t]] \quad (1)$$

where $[\text{CI-981}]_o$ is the initial concentration of CI-981 and [Lactone] is the concentration of the lactone formed at time t. As with the initial-rate method, lactone formation, instead of hydroxyacid disappearance, was followed because the kinetic results were less variable. Additionally, the pseudo-first-order rate constant for lactonization of the hydroxyacid, k_1, was obtained from the relationship,

$$K_{eq} = \frac{k_1}{k_2} \quad (2)$$

In borate and phosphate buffers, the lactone form was used as the starting material, and its conversion to the hydroxyacid form was followed to completion (i.e., total loss of the lactone), and k_2 was obtained from the slopes of plots of the natural log of the lactone peak area versus time. The exception was the kinetic run performed in pH 5.73 phosphate buffer where the lactone peak reached an equilibrium which was still detectable by HPLC; the rate and equilibrium constants were obtained by fitting the hydroxyacid formation data to appropriately modified versions of Eqs. (1) and (2). Under the conditions studied, the only reaction occurring appeared to be lactone formation or hydrolysis.

The apparent activation parameters for the specific-acid catalyzed pathways for both lactone formation and hydrolysis were determined from the effect of temperature (ranging from 20 to 50°C) on the k_1^H and k_2^H [described in Eqs. (4) and (5)] values calculated in 50 m*M* HCl (pH 1.3), whereas the apparent activation parameters for the hydroxide ion-catalyzed pathway for the lactone hydrolysis reaction were determined from the effect of temperature (ranging from 10 to 30°C) of the k_2^{OH} [described in Eq. (5)] value calculated in 100 m*M*, pH 9.0 borate buffer.

RESULTS AND DISCUSSION

Solubility Studies

Figure 2 shows the apparent solubility at 30°C of the sodium salt of CI-981 as a function of pH. Over the pH range of 2.1 to 6.0, the solubility increases about 60-fold, from 20.4 μg/mL to 1.23 mg/mL. The shape of the profile is consistent with ionization of the carboxyl group, and the theoretical profile in Fig. 2 was generated (using PCNONLIN) with an intrinsic solubility of 30.5 μg/mL for the free-acid form and a K_a of 3.47×10^{-5} (or a pK_a of 4.46). The apparent solubility of the lactone form of CI-981 varied little over the pH range of 2.3 to 7.8; the mean solubility over this range was 1.34 (±0.53) μg/mL. For both studies, the alternate form of CI-981 was present, and its effect on the reported solubility

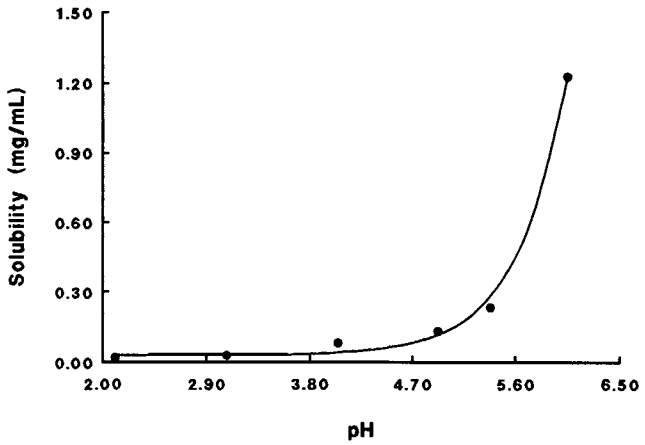


Fig. 2. The pH dependence of the apparent solubility of the sodium salt of CI-981 at 30°C. The theoretical profile was generated with an intrinsic solubility of 3.05×10^{-2} mg/mL and a K_a of 3.47×10^{-5}.

values is unknown. However, the presence of varying concentrations of the hydroxyacid form did not appear to alter the solubility of the lactone form, suggesting the absence of complex formation between the two forms (6).

pH–k_{obs} Profiles

The kinetics of interconversion and the equilibrium between the hydroxyacid and the lactone forms were studied, with a stability-indicating HPLC assay, as a function of pH, buffer concentration, and temperature at an ionic strength of 0.5 *M*. The equilibrium is depicted as

$$\text{Hydroxyacid} \underset{k_2}{\overset{k_1}{\rightleftarrows}} \text{Lactone} + H_2O \qquad (3)$$

Table I shows the dependence of the observed rate constants for lactone formation and hydrolysis, k_1 and k_2, and the associated equilibrium constant, K_{eq}, on pH. Figure 3 depicts the dependence graphically for k_1 and k_2. (Although the simplified reaction scheme shows k_2 as a second-order rate constant, the contribution of the water term was not factored out, hence the reported k_2 values are pseudo-first-order rate constants.)

The dependence of k_1 on the hydrogen-ion activity, a_H, can be described by the following expression:

$$k_1 = k_1^H a_H f_N + k_1^O f_A \qquad (4)$$

where K_a is the ionization constant for the terminal carboxyl group of the hydroxyacid form; k_1^H and k_1^O are the rate constants for specific acid-catalyzed lactonization of the neutral form and spontaneous lactonization of the anionic form of the hydroxy acid, respectively; and f_N and f_A are the fractions of the hydroxyacid existing in the neutral and anionic forms, respectively. Substituting for these latter terms results in

Table I. The pH Dependencies of the Rate Constants for Lactone Formation (k_1) and Hydrolysis (k_2) and the Associated Equilibrium Constant (K_{eq}) at 80°C and μ = 0.5 *M*

Reaction medium	pH	k_1 (hr^{-1})	k_2 (hr^{-1})	K_{eq}
HCl	1.30	13.6[a]	20.2[a]	0.673[a]
HCl	1.64	5.62	7.67	0.733
HCl	1.98	2.57	3.36	0.766
Formate buffer	3.00	2.28×10^{-1}	3.42 ± 10^{-1}	0.667
Formate buffer	3.50	8.80×10^{-2}	1.51×10^{-1}	0.583
Formate buffer	4.00	2.98×10^{-2}	5.13×10^{-2}	0.581
Acetate buffer	4.12	2.66×10^{-2}	5.64×10^{-2}	0.472
Acetate buffer	4.60	1.39×10^{-2}	5.59×10^{-2}	0.248
Acetate buffer	5.10	1.15×10^{-2}	1.02×10^{-1}	0.113
Phosphate buffer	5.73	1.51×10^{-2}	5.16×10^{-1}	0.029
Phosphate buffer	6.32	—	1.41	—
Phosphate buffer	6.86	—	5.20	—
Borate buffer	8.48	—	123[a,b]	—
Borate buffer	8.70	—	214[a,b]	—
Borate buffer	9.00	—	372[a,b]	—

[a] Extrapolated from the corresponding Arrhenius relationship. [b] Buffer catalysis was observed, and these values represent the buffer-independent rate constants.

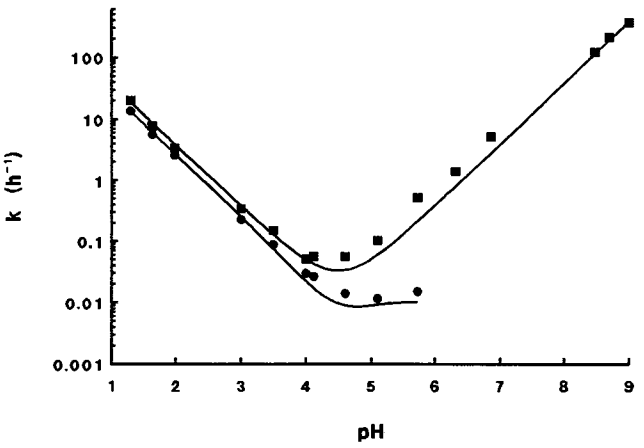


Fig. 3. The pH dependence of the rate constants for lactone formation, k_1 (●), and hydrolysis, k_2 (■), at 80°C and an ionic strength of 0.5 *M*. The theoretical profiles were calculated with Eqs. (5) and (6) and the constants shown in the text.

$$k_1 = k_1^H a_H \left(\frac{a_H}{a_H + K_a}\right) + k_1^O \left(\frac{K_a}{a_H + K_a}\right) \qquad (5)$$

The theoretical profile for k_1, shown in Fig. 3, was generated with a k_1^H of of 266 (±6) M^{-1} hr^{-1}, a k_1^O of 1.08×10^{-2} ($\pm 1.52 \times 10^{-1}$) hr^{-1}, and a K_a of 3.47×10^{-5} ($\pm 5.54 \times 10^{-4}$) or a pK_a of 4.46. This pK_a value is consistent with the value obtained from the solubility data and with simple carboxylic acids having pK_a values that are relatively insensitive to temperature changes (7).

The dependence of k_2 on a_H can be described by the following expression:

$$k_2 = k_2^H a_H + k_2^O + k_2^{OH} \frac{K_w}{a_H} \qquad (6)$$

where K_w is the ion product of water and k_2^H, k_2^O, and k_2^{OH} are the rate constants for specific acid-, water-, and specific base-catalyzed hydrolysis of the lactone form, respectively. The theoretical profile for k_2, shown in Fig. 3, was generated with a k_2^H of 384 (±147) M^{-1} hr^{-1}, a k_2^O of 8.99×10^{-3} (± 2.32) hr^{-1}, and a k_2^{OH} of 1.52×10^6 ($\pm 2.83 \times 10^4$) M^{-1} hr^{-1}.

Perturbations in the pH–rate profiles, at pH values less than the pK_a, for the lactonization of coumarinic acids have been attributed to a change in the rate-determining step with pH; the proposed mechanism involved a pH-dependent equilibrium between a cationic and a neutral tetrahedral intermediate (8,9). For CI-981, there is no readily discernable pertubation in the pH–rate profile at pH <pK_a, and hence there is no evidence for a change in the rate-determining step with pH. Similar results were noted for the lactonization of substituted *o*-hydroxyphenylpropionic acids (10).

Figure 4 shows the dependence of the observed equilibrium constant, K_{eq}, on pH; the line is the theoretical profile which was obtained by substituting Eqs. (4) and (5) into Eq. (2) and then solving for K_{eq} as a function of a_H. At pH <6, the equilibrium reaction is detectable, whereas at pH >6, the equilibrium reaction is no longer detectable although in theory it still pertains. Over the pH range of 1.3 to 3.0, the apparent equilibrium constant, K_{eq}, favors the hydroxyacid

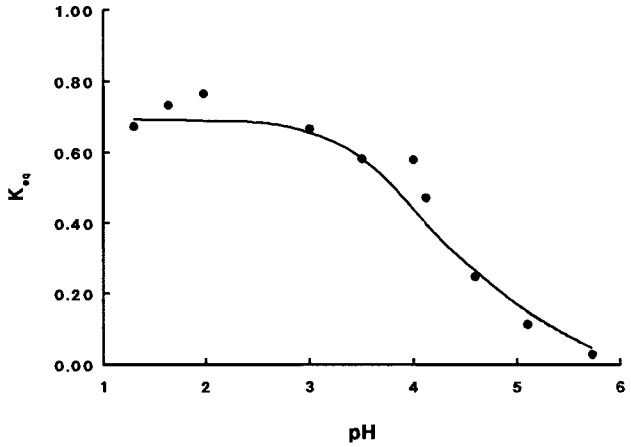


Fig. 4. The pH dependence of the apparent equilibrium constant at 80°C and an ionic strength of 0.5 *M*. The theoretical profile was calculated as described in the text.

form and is independent of pH. The latter finding is consistent with the parallel behavior of k_1 and k_2 in this pH range (Fig. 3), where the primary contributors to both rate constants are the respective specific acid-catalyzed terms, k_1^H and k_2^H. In contrast, at pH >3, K_{eq} becomes pH dependent; approaches zero; and, hence, increasingly favors the hydroxyacid over the lactone form. This is consistent with the divergence of k_1 and k_2, where k_2 becomes increasingly greater than k_1 as the pH increases (Fig. 3). (This also explains why at pH >6 the disappearance of lactone can be treated as an irreversible reaction.)

In addition to CI-981, the hydroxyacid form is increasingly favored as the pH increases from neutrality for other compounds susceptible to lactone formation and hydrolysis reactions. However, these compounds can show pronounced differences in their equilibrium values in acidic pH. For example, unlike CI-981, (*S*)-9-dimethylaminomethyl-10-hydroxycamptothecin exists exclusively in its lactone form at pH ≤4 (11), and likewise, camptothecin apparently was not susceptible to specific-acid catalyzed lactone hydrolysis (12).

Buffer Effects

Altering the concentrations of the formate, acetate, and phosphate buffers had no appreciable effect on the rate constants, consistent with the absence of buffer catalysis. In contrast, borate buffers catalyzed the rate of lactone hydrolysis (denoted k_2 or, more specifically, k_2^{OH}). The reaction appeared to be subject to both general-acid and -base catalysis, although the base catalysis was the more important of the two processes. The buffer-dependent rate constants were 2.14, 8.32, and 11.3 M^{-1} hr^{-1} at pH 8.46, 8.80, and 9.00, respectively.

Mechanisms of Interconversion

Lactone formation and hydrolysis reactions have been well characterized in the chemical literature (e.g., Refs. 8, 10, 13–15), and the established mechanisms should apply to CI-981 since the reactive moiety is a simple, unhindered, dihydroxy carboxylic acid (Fig. 1).

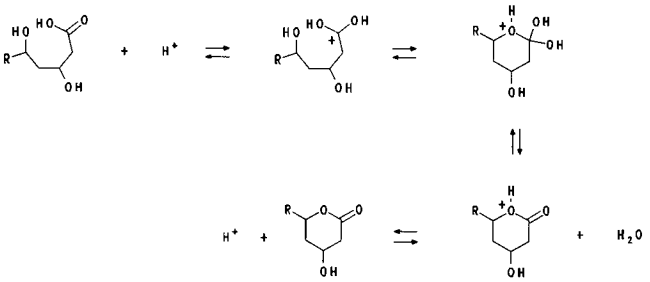
The postulated mechanism for specific acid-catalyzed lactone formation, k_1^H, involves intramolecular, nucleophilic catalysis by the δ-hydroxyl group and involves the formation of a tetrahedral intermediate (Scheme I). In addition to being reversible, the reaction is symmetrical, meaning that the mechanism for specific acid-catalyzed lactone hydrolysis, k_2^H, is the reverse reaction. The latter reaction involves nucleophilic participation by a water molecule and also proceeds via a tetrahedral intermediate; it has been classified as an AAC2 mechanism with the Ingold classification system (16).

The postulated mechanism for specific base-catalyzed lactone hydrolysis, k_2^{OH}, involves nucleophilic participation by hydroxide ion, resulting in acyl cleavage via a tetrahedral intermediate; it has been classified as a BAC2 mechanism with the Ingold classification system. For CI-981, this reaction can essentially be treated as an irreversible reaction at pH >6 even though, in theory, it is reversible.

Temperature Effects

Figure 5 shows the Eyring plots for specific acid-catalyzed lactone formation and hydrolysis and for specific base-catalyzed lactone hydrolysis. The associated activation energies (E_a), free energies ($\Delta G\ddagger$), enthalpies ($\Delta H\ddagger$), and entropies ($\Delta S\ddagger$) are shown in Table II. The values found for the k_1^H pathway are very similar to the values for the k_2^H pathway, implying a K_{eq} value, in the associated region of the profile, that more closely approximates 0.5 than either 1 or 0. The k_1^H values are also in good agreement with those found for other lactonization reactions (e.g., Ref. 17). For example, the acid-catalyzed lactonization of γ-hydroxybutanoic acid had a $\Delta H\ddagger$ of 12.06 kcal/mol and a $\Delta S\ddagger$ of −31.5 eu. The temperature dependence of the k_2^{OH} pathway was performed in 100 m*M* borate buffer. Since borate buffers catalyzed this pathway, the reported values do not represent the buffer-independent activation parameters, and must be interpreted cautiously.

With the corresponding E_a values, the rates of interconversion at 25°C in the specific acid- and -base catalyzed regions can be estimated. In the k_1^H controlled region (over a pH range of 1 to 3.5), the rate will be 50-fold slower at 25 than at 80°C; the estimated $t_{1/2}$ at 25°C varies from 2.6 hr at pH 1 to 16.5 days at pH 3.5. In the k_2^H controlled region (same pH range as for k_1^H), the rate will be 39-fold slower at 25°C; the estimated $t_{1/2}$ at 25°C varies from 1.3 hr at pH 1 to



Scheme I. Proposed mechanism for the specific acid-catalyzed lactonization of the hydroxyacid form of CI-981 (forward direction) and the specific acid-catalyzed hydrolysis of the lactone of CI-981 (reverse direction). R represents the remainder of the molecule.

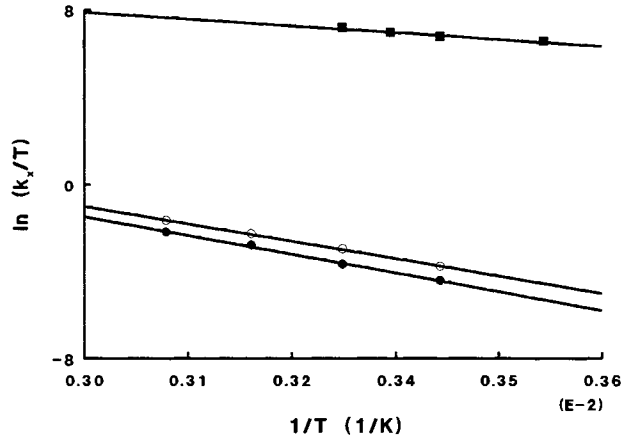


Fig. 5. The Eyring plots for the k_1^H (●), k_2^H (○), and k_2^{OH} (■) pathways. The rate constants are hr^{-1}.

7.5 days at pH 3.5. Finally, in the k_2^{OH} controlled region (over the pH range of 5.7 to 9.0), the rate will be 168-fold slower at 25°C; the estimated $t_{1/2}$ at 25°C varies from 9.4 days at pH 5.7 to 19 min at pH 9.0.

At pH <3, where K_{eq} is pH independent, the equilibrium is shifted toward the hydroxyacid form as the temperature is decreased; the theoretical, kinetically generated K_{eq} goes from 0.69 at 80°C to 0.51 at 25°C. This results from the larger E_a associated with the k_1^H than with the k_2^H pathway. The theoretical, thermodynamically generated K_{eq} is the same as the kinetically generated value at 25°C (0.51) but is slightly less at 80°C (0.57). The thermodynamic values were calculated with the free energy difference between the hydroxyacid form (the reactant state) and the lactone form (the product state), which is 0.4 kcal/mol (from Table II). Although the E_a values were not determined for the k_1^O and the k_2^O pathways (because of the much slower rates in the pH regions where these constants are significant contributors to the overall rate constant), it is expected that at 25°C and at pH ≳6, the equilibrium greatly favors the hydroxyacid form as observed at 80°C.

CONCLUSIONS

When the hydroxyacid form of CI-981 exists as the free acid species, it is about 15 times more soluble than the lactone form of CI-981. As the carboxyl group of the hydroxyacid ionizes, this difference in the solubility becomes even greater. The specific acid-catalyzed reaction is reversible, whereas the specific base-catalyzed reaction can be treated as an irreversible reaction at pH >6. More specifically, an equilibrium, between the hydroxyacid and the corresponding lactone form, which favors the hydroxyacid form, occurs at pH <6, whereas at pH >6 the equilibrium greatly favors the pharmacologically active hydroxyacid form. The biologic differences between the hydroxyacid and the lactone forms of CI-981 (and attempts to correlate these differences with the physicochemical properties discussed here) are currently being studied.

Table II. The Apparent Activation Parameters for Specific Acid-Catalyzed Lactone Formation (k_1^H) and Hydrolysis (k_2^H) and for Specific Base-Catalyzed Lactone Hydrolysis (k_2^{OH})[a]

Reaction pathway	E_a (kcal/mol)	$\Delta G‡$ (kcal/mol)[b]	$\Delta S‡$ (eu)[c]	$\Delta H‡$ (kcal/mol)[c]
k_1^H	14.9 ± 0.6	21.3	−23.5 ± 2.0	14.3 ± 0.6
k_2^H	13.9 ± 0.3	20.9	−25.5 ± 0.9	13.3 ± 0.3
k_2^{OH}	5.7 ± 0.8	14.8	−32.4 ± 2.8	5.1 ± 0.8

[a] The errors are standard errors.
[b] Obtained from $\Delta G‡ = \Delta H‡ - T\Delta S‡$ at 25°C.
[c] Obtained from $\ln(k/T) = 31.95 + (\Delta S‡/1.987) - [\Delta H‡/(1.987 \cdot T)]$.

REFERENCES

1. S. M. Grundy. HMG-CoA reductase inhibitors for treatment of hypercholesterolemia. *N. Engl. J. Med.* 319:24–32 (1988).
2. B. D. Roth, D. R. Sliskovic, and B. K. Trivedi. Treatment of hypercholesterolemia. *Ann. Rep. Med. Chem.* 24:147–156 (1989).
3. M. K. Shaw, R. S. Newton, D. R. Sliskovic, B. D. Roth, E. Ferguson, and B. R. Krause. Hep-G2 cells and primary rat hepatocytes differ in their response to inhibitors of HMG-CoA reductase. *Biochem. Biophys. Res. Commun.* 170:726–734 (1990).
4. B. D. Roth, C. J. Blankley, A. W. Chucholowski, E. Ferguson, M. L. Hoefle, D. F. Ortwine, R. S. Newton, C. S. Sekerke, D. R. Sliskovic, C. D. Stratton, and M. W. Wilson. Inhibitors of cholesterol biosynthesis. 3. Tetrahydro-4-hydroxy-6-[2-(1H-pyrrol-1-yl)ethyl]-2H-pyran-2-one inhibitors of HMG-CoA reductase. 2. Effects of introducing substituents at positions three and four of the pyrrole nucleus. *J. Med. Chem.* 34:357–366 (1991).
5. K. L. Baumann, D. E. Butler, C. F. Deering, K. E. Mennen, A. Millar, T. N. Nanninga, C. W. Palmer, and B. D. Roth. The convergent synthesis of CI-981, an optically active, highly potent, tissue selective inhibitor of HMG-CoA reductase. *Tetrahedron Lett.* 33:2283–2284 (1992).
6. A. J. Repta. Alteration of apparent solubility through complexation. In S. H. Yalkowsky (ed.), *Techniques of Solubilization of Drugs*, Marcel Dekker, New York, 1981, pp. 135–157.
7. D. D. Perrin, B. Dempsey, and E. P. Serjeant. *pK_a Prediction for Organic Acids and Bases*, Chapman and Hall, New York, 1981, pp. 7–9.
8. R. Hershfield and G. L. Schmir. The lactonization of ring-substituted coumarinic acids. Structural effects on the partitioning of tetrahedral intermediates in esterification. *J. Am. Chem. Soc.* 95:7359–7369 (1973).
9. E. R. Garrett, B. C. Lippold, and J. B. Mielck, Kinetics and mechanisms of lactonization of coumarinic acids and hydrolysis of coumarins I. *J. Pharm. Sci.* 60:396–405 (1971).
10. S. Milstien and L. A. Cohen. Concurrent general-acid and general-base catalysis of esterification. *J. Am. Chem. Sco.* 92: 4377–4382 (1970).
11. W. J. M. Underberg, R. M. J. Goossen, B. R. Smith, and J. H. Beijnen. Equilibrium kinetics of the new experimental antitumour compound SK&F 104864-A in aqueous solution. *J. Pharm. Biomed. Anal.* 8:681–683 (1990).
12. J. Fassberg and V. J. Stella. A kinetic and mechanistic study of the hydrolysis of camptothecin and some analogues. *J. Pharm. Sci.* 81:676–684 (1992).
13. D. R. Storm and D. E. Koshland. Effect of small changes in orientation on reaction rate. *J. Am. Chem. Soc.* 94:5815–5825 (1972).
14. E. T. Kaiser and F. J. Kézdy. Hydrolysis of cyclic esters. *Prog. Bioorg. Chem.* 4:239–267 (1976).
15. B. Capon and S. P. McManus. *Neighboring Group Participation, Vol. 1*, Plenum Press, New York, 1976, p. 183.
16. C. K. Ingold. *Structure and Mechanism in Organic Chemistry*, 2nd ed., Cornell University Press, Ithaca, NY, 1969, pp. 1129–1131.
17. D. R. Storm and D. E. Koshland. An indication of the magnitude of orientation factors in esterification. *J. Am. Chem. Soc.* 94:5805–5814 (1972).