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-pyroline-1-hepatic acid) is an important consideration when choosing 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 pKs 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, k1, is well described by specific acid-catalyzed and spontaneous lactonization pathways, whereas the rate constant for lactone hydrolysis (or hydroxyacid formation), k2, 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-pyroline-1-hepatic 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 choosing 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 mM 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 (tR) of about 4 min, whereas the lactone form had a tR 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 polystyrene 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...
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 mM) 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:

\[
[Lactone] = \frac{K_{eq} \cdot [CI-981]_0}{(K_{eq} + 1)} \cdot \left[1 - \exp[-(k_2 + K_{eq} \cdot k_2)t]\right]
\]  

where \([CI-981]_0\) 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,

\[
K_{eq} = \frac{k_1}{k_2}
\]  

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 mM 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 mM, 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 p\( K_a \) 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...