Aqueous Stability and Solubility of CI-988, a Novel "Dipeptoid" Cholecystokinin-B Receptor Antagonist

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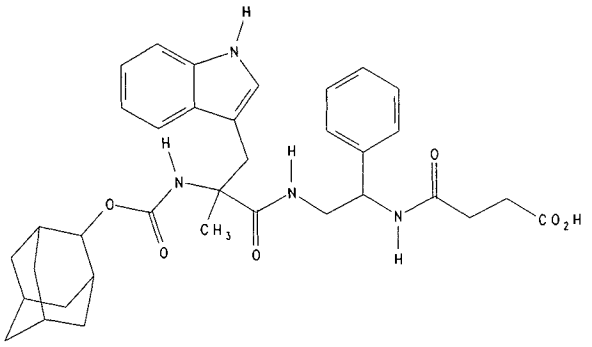
The aqueous solubility and solution stability of the *N*-methylglucamine and sodium salts of CI-988 (CI-988 NMG and CI-988 Na) were evaluated to aid in the development of a parenteral formulation for preclinical and clinical testing. CI-988 ([*R*-(*R**,*R**)]-4-[[2-[[3-(1*H*-indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo[3.3.1.1^{3,7}]dec-2-yloxy)carbonyl]amino]propyl]amino]-1-phenylethyl]amino]-4-oxobutanoic acid) is a selective "dipeptoid" cholecystokinin-B receptor antagonist. The shape of the pH–solubility profile, generated at 30°C, is consistent with the ionization of the terminal carboxyl group (pK_a of 4.34). The pH–rate profile is independent of the salt form and is well described by two reaction pathways: spontaneous or water-catalyzed degradation of the nonionized form and specific base-catalyzed degradation of the ionized form. The primary mechanism of degradation from the former pathway is consistent with intramolecular, carboxyl-assisted, amide-bond cleavage, whereas the primary mechanism of degradation from the latter pathway appears to be intramolecular cyclization to a hydantoin product with expulsion of 2-adamantanol. The pH dependencies of the solubility and stability show that a simple aqueous buffered solution of CI-988 has a predicted t_{90} of 2.1 years and a solubility of 0.94 mg/ml at pH 6.5, the theoretical pH of maximum stability, and 30°C.

KEY WORDS: aqueous solubility; solution stability; cholecystokinin-B; antagonist; amide bond hydrolysis; hydantoin formation.

INTRODUCTION

Cholecystokinin (CCK), an endogenous mammalian peptide and member of the gastrin family, is present at high concentrations in the CNS (1,2). It has been postulated to act as either a neurotransmitter or a neuromodulator (3), hence CCK-specific receptors might represent novel chemotherapeutic targets.



CI-988([*R*-(*R**,*R**)]-4-[[2-[[3-(1*H*-indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo[3.3.1.1^{3,7}]dec-2-yloxy)carbonyl]amino]propyl]amino]-1-phenylethyl]amino]-4-oxobutanoic acid), currently in Phase 1 clinical trials, is a "dipeptoid" analogue of the C-terminal tetrapeptide, residues 30–33, of CCK. It is a selective antagonist having a nanomolar affinity for the subtype B cholecystokinin receptors that are widely distributed throughout the brain (4). Pharmacologically, it is a potent, orally active, and nonsedating anxiolytic in mice, and it lacks withdrawal anxiogenesis following cessation of treatment and also produces an anxiolytic effect in mice previously made tolerant to diazepam (5).

The solubility and solution stability of the *N*-methylglucamine and sodium salts of CI-988 (CI-988 NMG and CI-988 Na) in an aqueous environment were investigated as a function of pH to aid in its preclinical and clinical evaluation and its development as a potential drug candidate.

MATERIALS AND METHODS

Materials

CI-988 NMG, CI-988 Na, and the primary degradation product in the acidic pH region were synthesized by Parke-Davis Research. All other chemicals were of reagent or analytical grade, and the water was distilled and deionized.

Analytical Methods

The pH measurements were performed with an Accumet pH meter 925 and a Ross combination glass electrode. 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 220 nm. The column was a Supelcosil LC-CN (4.6 mm × 25-cm) 5-μm column. The mobile phase was comprised of a 50:50 mixture of acetonitrile:25 m*M* NaH_2PO_4 in water. The injection volume was 20 μl, and the eluent flow rate was 1.5 ml/min.

Solubility Determination

The aqueous solubility of CI-988 Na was determined as a function of pH at 30°C. The pH was maintained with 10 and 50 m*M* HCl (pH 2.04 and 1.41, respectively) and with 50 m*M* acetate (pH 4.23–5.19) and phosphate (pH 6.48 and 6.78) buffers, which were made with the potassium salts to eliminate a common-ion effect. The phosphate buffer at pH 6.78 had to be adjusted with HCl because it did not have sufficient buffer capacity. Excess solid CI-988 NMG or CI-988 Na was equilibrated, using a rotating-bottle apparatus, in the medium of interest for at least 48 hr before filtration through a 0.45-μm syringe filter (Gelman Acrodisc CR PTFE). (The equilibrium solubility was attained within 24 hr.) About 4 ml of the mixture was required to saturate the filter with drug. Following filter saturation, the filtrate was collected and used for solubility and pH determinations. The samples in the phosphate buffers were diluted before they were assayed.

Kinetic Methods

The kinetics of degradation of dilute aqueous solutions of CI-988 NMG and CI-988 Na (1.5×10^{-5} *M*) were deter-

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mined as a function of pH. For CI-988 Na, the kinetics were also determined as a function of buffer concentration (i.e., 25, 50, 75, and 100 m*M*) and temperature at $\mu = 0.5$ *M* with NaCl. The buffer species used were formate, acetate, phosphate, and borate. Kinetic experiments were started by adding 0.10 ml of a stock solution of CI-988 NMG or CI-988 Na to 10-ml volumetric flasks of the reaction mixtures, which were temperature equilibrated in a circulating-water bath. The pH's of these mixtures were measured at the temperature of the study at the end of each kinetic run.

At appropriate time intervals, samples were withdrawn, quenched in an ice-water bath, and assayed for CI-988 and degradants by HPLC. The observed rate constants, k_{obs}, were obtained by following the disappearance of the peak area of CI-988 for at least two half-lives.

The rate constants comprising k_{obs} were generated by nonlinear least-squares regression of Eq. (1) and the experimental data using PCNONLIN (SCI, Lexington, KY) and the Nelder–Mead simplex algorithm.

RESULTS AND DISCUSSION

Solubility Determinations

The increase in the aqueous solubility which can be achieved through ionization of CI-988 was determined by generating a pH–solubility profile. As shown in Fig. 1, the solubility of CI-988 Na increases about 4000-fold, from 5.9×10^{-4} to 2.4 mg/ml, over the pH range of 1.4 to 6.8. Also, CI-988 NMG and CI-988 Na are freely soluble in water, and the pH of the resulting solution is basic. (The solubility of CI-988 NMG in water is greater than 200 mg/ml, and the pH of a 200 mg/ml solution is 8.2.) The shape of the profile is consistent with the ionization of the terminal carboxyl group; a pK_a value of 4.34 ± 0.21 (uncorrected for activity coefficients) is obtained, by standard techniques (6), from the solubility data in the acetate buffers ($n = 6$). The theoretical solubility profile in Fig. 1 is generated with the above pK_a and an intrinsic solubility of 5.9×10^{-4} mg/ml for the nonionized form. Deviation of the solubilities in the 50 m*M* phosphate buffers from the theoretical profile may be due to self-association of the ionized form of CI-988 and to changes in the activity coefficients.

Examination of the pH–Rate Profile

The kinetics of hydrolysis of dilute aqueous solutions of CI-988 NMG and CI-988 Na were studied as a function of pH. For CI-988 Na, the kinetics were also studied as a function of buffer concentration and temperature at $\mu = 0.5$ *M* (with NaCl) by HPLC. The kinetics under all conditions were well described as first-order processes. Figure 2 shows the dependence of the degradation rate constants, k_{obs}, on the pH at 80°C. Altering the concentration of the formate, acetate, phosphate, or borate buffer at a fixed pH had little to no effect on the degradation rate, consistent with the lack of buffer catalysis. Hence, the k_{obs} values are the means of the values found at four different buffer concentrations. The pH–rate profile exhibited a minimum at about pH 6.0 and a pH-independent region at pH less than 3.

Over the pH range investigated, an expression, relating the observed first-order rate constant to the hydrogen-ion activity, can be derived:

$$k_{obs} = \left(k_O a_H + k_{OH} K_a \frac{K_w}{a_H}\right)\left(\frac{1}{a_H + K_a}\right) \quad (1)$$

where a_H is the hydrogen-ion activity, K_a is the ionization constant of the terminal carboxyl group, K_w is the ion product of water, k_O is the rate constant for spontaneous or water-catalyzed degradation of the free acid, and k_{OH} is the rate constant for the hydroxide ion-catalyzed degradation of the anion. The reaction pathway involving water-catalyzed or spontaneous degradation of the free acid is kinetically equivalent to a pathway involving hydronium ion-catalyzed degradation of the anion, k_H.

The theoretical profile in Fig. 2 is constructed with a k_O of 5.45 (±0.66) $\times 10^{-2}$ hr^{-1}, a k_{OH} of 1700 (±3) M^{-1} hr^{-1}, and a K_a of 6.31×10^{-5}. The pK_a of 4.20 at 80°C is in good agreement with the value of 4.34 determined at 30°C by the solubility method, and this finding is consistent with the fact that the pK_a values of simple carboxylic acids are relatively insensitive to temperature changes (7). The same theoretical

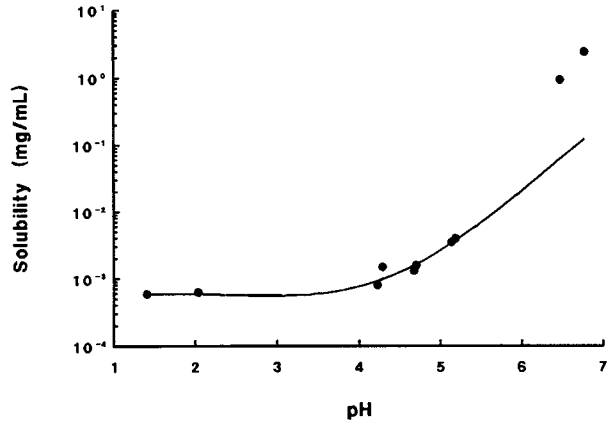


Fig. 1. Solubility of CI-988 Na as a function of pH at 30°C. The line is the theoretical profile based on a pK_a of 4.34 and an intrinsic solubility of 5.9×10^{-4} mg/ml for the free acid.

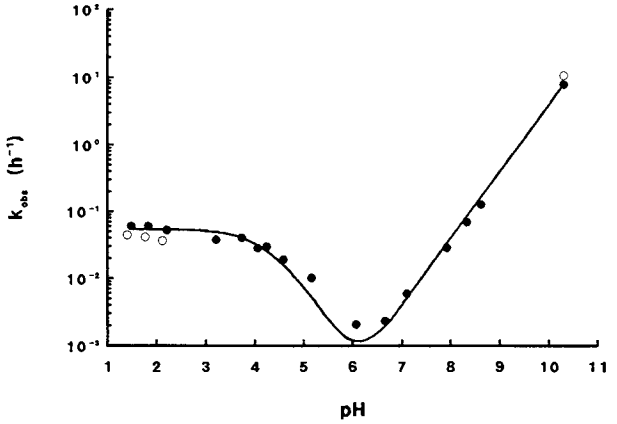


Fig. 2. pH–rate profiles for the degradation of CI-988 NMG (○) and CI-988 Na (●) at 80°C and $\mu = 0.5$ *M* (with NaCl). The line represents the theoretical profile generated with nonlinear least-squares regression of the experimental data (●) using Eq. (2).

profile can be generated with a k_H of 863 M^{-1} hr^{-1} instead of the kinetically equivalent k_O. Additionally, the profile was independent of the salt form used (i.e., CI-988 NMG or CI-988 Na).

The apparent activation parameters for the two distinct degradation pathways were determined from the effect of temperature on k_O and k_{OH} for CI-988 Na (although the same parameters would be expected for CI-988 NMG). Figure 3 shows the corresponding Arrhenius plots. The k_O values are the averages of the rate constants (k_{obs}) determined in 10, 25, and 50 mM HCl (the pH-independent region of the pH–rate profile). For this pathway, the energy of activation (E_O) was 21.3 ± 3.7 kcal/mol; the enthalpy of activation ($\Delta H^{\neq}$) was 20.6 ± 3.7 kcal/mol, and the entropy of activation ($\Delta S^{\neq}$) was −22.1 eu at 30°C. The k_{OH} values were calculated from the k_{obs} values determined in 10 mM NaOH and the K_w values at the temperatures of interest. For this pathway, the energy of activation (E_{OH}) was 15.4 ± 2.2 kcal/mol, $\Delta H^{\neq}$ was 14.7 ± 2.3 kcal/mol, and $\Delta S^{\neq}$ was −17.8 eu at 30°C.

The pH–rate profile can be predicted at any temperature by substituting the appropriate Arrhenius equations into Eq. (1), which results in

$$k_{obs} = [A_O a_H \exp(-E_O/RT) + A_{OH} K_a \frac{K_w}{a_H} \exp(-E_{OH}/RT)] \left(\frac{1}{a_H + K_a}\right) \quad (2)$$

where R is the gas constant, T is the temperature in Kelvin, and A_O and A_{OH} are the Arrhenius frequency factors for water-catalyzed or spontaneous degradation of the free acid and hydroxide ion-catalyzed decomposition of the anion, respectively. The apparent t_{90} value in the pH-independent region (pH <3) is about 11 days, and it is 2.1 years at pH 6.5, the theoretical pH of maximum stability, and at 30°C.

Mechanisms of Degradation

The primary products of degradation are different for the two reaction pathways. For the spontaneous or water-catalyzed pathway, the predominant degradation reaction appears to be intramolecular, carboxyl-facilitated, amide-bond cleavage. This is substantiated by comparison of UV spectra and coelution on HPLC with an authentic sample of the product. A possible mechanism (Scheme I) is the one

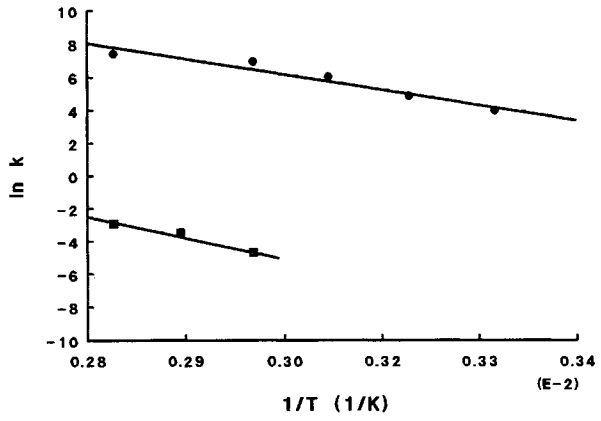
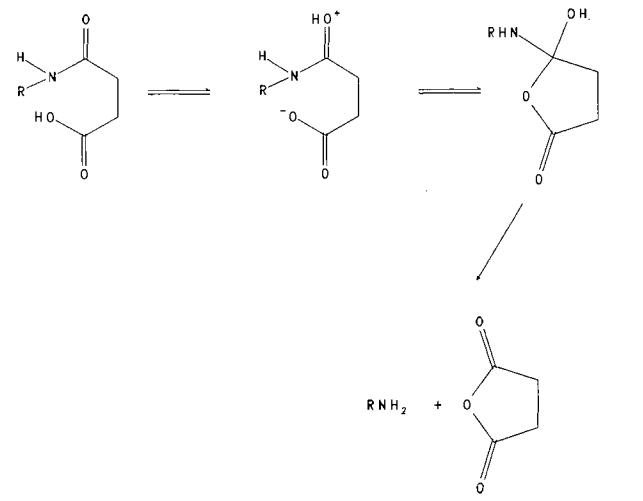


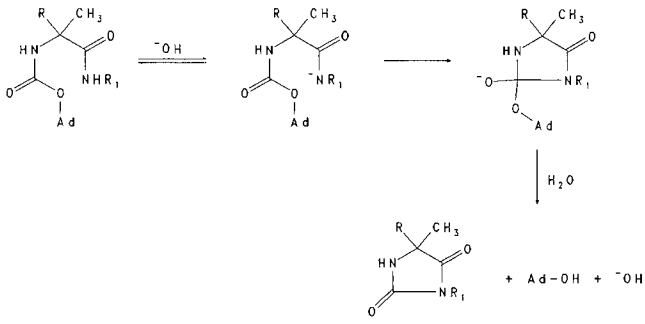
Fig. 3. Arrhenius plots of k_O (■) and k_{OH} (●) for the degradation of CI-988 Na at μ = 0.5 M (with NaCl).



Scheme I. R represents the remainder of the CI-988 molecule.

previously proposed for the intramolecular, carboxyl-assisted hydrolysis of structurally analogous amides (8–14). It involves transfer of the carboxyl proton to the oxygen atom of the amide group either prior to or concerted with nucleophilic attack by the carboxylate group at the carbonyl carbon atom of the amide leading to the formation of a tetrahedral intermediate, which would subsequently decompose to succinic anhydride and the terminal amine-containing compound (Scheme I). The mechanism has been depicted as involving intramolecular nucleophilic catalysis by the terminal carboxyl group based on literature precedent (8–14), but at least one other kinetically equivalent reaction, not involving a succinic anhydride intermediate, exists: intramolecular general-acid catalysis of attack by water.

For the hydroxide ion-catalyzed pathway, the predominant degradation reaction appears to be intramolecular cyclization to a hydantoin product with expulsion of 2-adamantanol. This reaction is supported by the occurrence of hydantoin formation, in basic aqueous-methanolic solutions, of a series of tryptophanylphenethylamides which are structurally analogous to CI-988 (15). A possible mechanism (Scheme II) involves a preequilibrium proton transfer from the amide nitrogen to the hydroxide ion, with subsequent nucleophilic attack by this nitrogen at the neighboring carbon atom of the carbamate moiety. This results in the formation of a tetrahedral intermediate which would subsequently break down to form a hydantoin derivative and 2-ad-



Scheme II. Ad is adamantane, and R and R_1 represent the remainder of the CI-988 molecule.

amantanol. Hydantoin formation in the basic, but not in the acidic, pH regions is consistent with the finding that nucleophilic catalysis by amide groups usually occurs by a mechanism involving hydroxide ion-catalyzed deprotonation of the amide nitrogen (16).

CONCLUSIONS

The pH dependencies of the solubility and solution stability show that a simple aqueous buffered solution of CI-988 has a predicted t_{90} of 2.1 years and a solubility of 0.94 mg/ml at pH 6.5, the theoretical pH of maximum stability, and 30°C. Currently, we are investigating ways of increasing the aqueous solubility of CI-988 and ways of stabilizing CI-988, with our knowledge of the degradation mechanisms, by an analogue approach.

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