International Journal of Pharmaceutics, 92 (1993) 63–70

IJP 03037

The effect of structural changes on the intramolecular degradation of the 'dipeptoid' CI-988

Albert S. Kearney, Surendra C. Mehta and Galen W. Radebaugh

Department of Pharmaceutics, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Morris Plains, NJ (USA)

(Received 13 July 1992)
(Modified version received 8 September 1992)
(Accepted 9 September 1992)

Key words: Stability; Cholecystokinin B antagonist; Analog; Amide bond hydrolysis; Intramolecular rearrangement

Summary

CI-988 ([*R*-(*R**,*R**)]-4-[[2-[[3-(1H-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. To further investigate its mechanisms of degradation (i.e., apparent carboxyl-assisted amide-bond cleavage in acidic pH and hydroxide-ion catalyzed hydantoin formation in basic pH (Kearney et al., *Pharm. Res.*, 9 (1992) 1095–1098), the kinetics of decomposition of three analogues of CI-988, differing only in the structure of the oxobutanoic acid side chain, were studied as a function of pH: **I**, [*R*-(*R**,*R**)]-2-[[[2-[[3-(1H-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]carbonyl]benzoic acid); **II**, [*R*-(*R**,*R**)]-5-[[2-[[3-(1H-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]-5-oxopentanoic acid); and **III**, [*R*-(*R**,*R**)]-[2-[(2-amino-2-phenylethyl)amino]-1-(1H-indol-3-ylmethyl)-1-methyl-2-oxoethyl]carbamic acid tricyclo[3.3.1.1^{3,7}]dec-2-yl ester. Alterations to this oxobutanoic acid side chain had little to no effect on the hydroxide-ion catalyzed pathway supporting a common degradation mechanism for all of the compounds. One exception was when **III** had a positive charge on the terminal amino; the cationic species degraded about 250-times faster than CI-988, **I**, **II**, or the corresponding neutral species of **III**. Alterations to the side chain had a dramatic effect on the carboxyl-assisted pathway: **I** degraded about 100-times faster than CI-988 via an entropically driven reaction, whereas **II** degraded about 17-times slower than CI-988 (although a different degradation mechanism(s) was involved). As expected, these latter results indicate that the rate of intramolecular reaction between the carboxyl and amide groups depends on the flexibility and length of the intervening carbon chain.

Introduction

Cholecystokinin (CCK)-specific receptors represent novel chemotherapeutic targets (Nadzan and Kerwin, 1991). CI-988 ([*R*-(*R**,*R**)]-4-[[2-[[3-(1H-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 'dipeptoid' antagonist which is highly selective for the CCK-B receptors that are widely distributed throughout the brain (Horwell et al., 1990). It produces anxiolytic effects in rodent and primate models of anxiety and is a potent, orally active,

Correspondence to: A.S. Kearney, Department of Pharmaceutics, Warner-Lambert Co., 170 Tabor Road, Morris Plains, NJ 07950, U.S.A.

and non-sedating anxiolytic in mice. Also, it lacks withdrawal anxiogenesis following cessation of treatment and produces an anxiolytic effect in diazepam-tolerant mice (Hughes et al., 1990).

Previously, CI-988 was postulated to degrade by two intramolecular mechanisms which affected two different parts of the molecule: carboxyl-assisted amide-bond cleavage in acidic pH and hydroxide-ion catalyzed hydantoin formation in basic pH (Kearney et al., 1992). In the present study, the kinetics of degradation, as a function of pH, of three analogues of CI-988, differing only in the structure of the oxobutanoic acid side chain (the reactive moiety in acidic pH), were investigated and compared. An analogue approach was chosen to allow us to further probe the degradation mechanisms while maintaining a fixed structural nucleus and also to provide a knowledge base to aid in evaluating the reactivity of potential back-up candidates from this series of compounds.

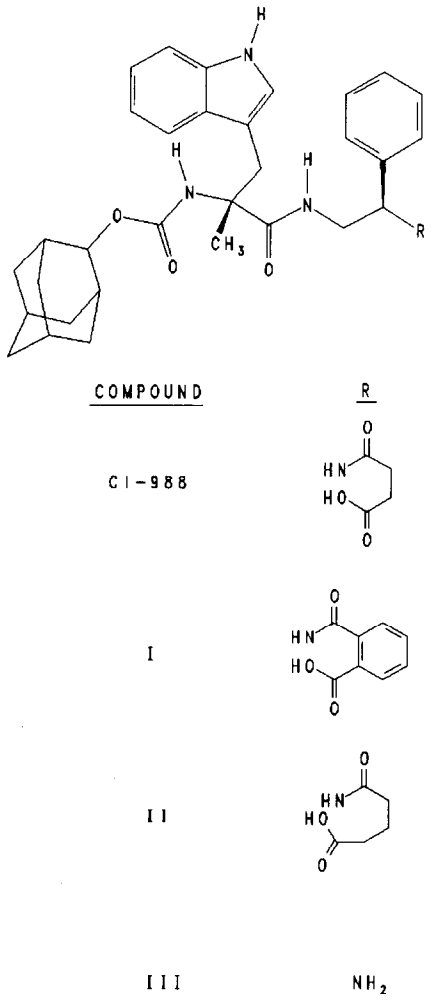
Materials and Methods

Materials

CI-988; the phthalamic acid analogue (**I**; [*R*-(*R**, *R**)]-2-[[[2-[[3-(1H-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]carbonyl]-benzoic acid); the pentanoic acid analogue (**II**; [*R*-(*R**,*R**)]-5-[[2-[[3-(1H-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]-5-oxo-pen-tanoic acid); and the analogue devoid of the oxobutanoic acid side chain (**III**; [*R*-(*R**,*R**)]-[2-[(2-amino-2-phenylethyl)amino]-1-(1H-indol-3-ylmethyl)-1-methyl-2-oxoethyl]carbamic acid tricyclo[3.3.1.1^{3,7}]dec-2-yl ester) were synthesized at Parke-Davis Research Unit in Cambridge, U.K. 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 using two-point standardization at the temperature of interest. The 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 a 50:50 mixture of acetonitrile:25 mM NaH_2PO_4 in water. The injection volume was 20 μl, and the eluent flow rate was 1.5 ml/min.

Kinetic methods

The kinetics of degradation of dilute aqueous solutions of **I–III** ($\sim 1.5 \times 10^{-5}$ M) were determined as a function of pH at 80°C and a fixed ionic strength of 0.5 M (with NaCl). When buffers were used, the kinetics were performed at a buffer

concentration of 50 mM. Kinetic experiments were initiated by adding 0.10 ml of a methanolic stock solution of a given analogue to 10-ml volumetric flasks containing reaction solutions which were temperature equilibrated in a circulating-water bath. For the degradation of **II** and **III** in acetate buffers, 1-ml aliquots of the reaction mixtures containing drug were flame-sealed in glass ampules and placed in a temperature-controlled oven. (This was done to prevent evaporation that may occur with the volumetric flask method, since a longer time period was required to reach two half-lives.)

At appropriate time intervals, 400-μl aliquots were withdrawn from the volumetric flasks, quenched in an ice-water bath, and assayed for **I**, **II**, or **III**, and for degradants by HPLC. For the samples sealed in ampules, ampules were periodically removed from the oven, quenched in an ice-water bath, and frozen until all samples were ready to be analyzed by HPLC. The pH of the reaction mixtures were measured at the temperature of the study and at the end of each kinetic run.

For CI-988 and **I**, the solvent-deuterium isotope effects for the k_0 pathway were determined by following the degradation kinetics in deuterium oxide under the conditions described above. The reaction mixtures were 25 and 50 mM HCl in deuterium oxide, and the pD was determined at 80°C by adding a factor of 0.4 to the pH

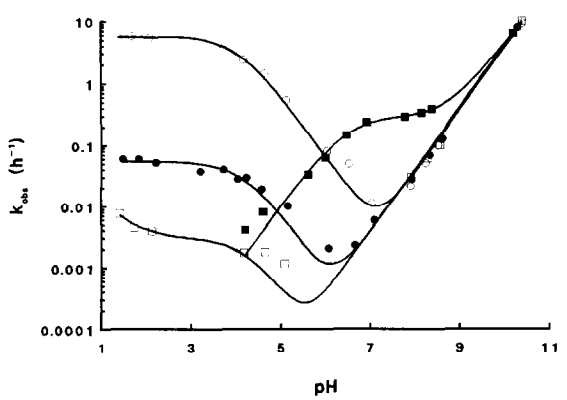


Fig. 1. pH-rate profiles for the degradation of CI-988 (●), **I** (○), **II** (□), and **III** (■) at 80°C and $\mu = 0.5$ M (with NaCl). The lines are the theoretical profiles generated with the rate constants from Table 1.

values (Glasoe and Long, 1960). Also, for CI-988 and **I**, the apparent activation parameters for the k_0 pathway were determined by following the degradation in 10, 25 and 50 mM HCl at 60, 70 and 80°C for CI-988 and at 50, 60, 70 and 80°C for **I**.

The observed rate constants, k_{obs}, were obtained by following the disappearance of the peak area of **I**, **II**, or **III** for at least two half-lives by HPLC. The rate constants comprising k_{obs} (i.e., k_H, k_0, and/or k_{OH}) were generated by an unweighted, non-linear, least-squares regression of an appropriate equation and the experimental data using PCNONLIN® (SCI, Lexington, KY) and the Nelder-Mead simplex algorithm.

Results and Discussion

Examination of the pH-rate profiles

The kinetics of degradation of dilute aqueous solutions of **I–III** were studied as a function of pH at 80°C and $\mu = 0.5$ M (with NaCl) by HPLC. When buffers were employed, a single buffer concentration of 50 mM was used because the degradation kinetics of CI-988, where similar degradation mechanisms were expected, were buffer independent (Kearney et al., 1992). The kinetics under all conditions were well described as first-order processes. Fig. 1 shows the dependence of the degradation rate constants, k_{obs}, on the pH for **I–III**, and CI-988. All profiles displayed an ascending region, having a slope of about unity, in basic pH, consistent with the involvement of a hydroxide-ion catalyzed pathway.

Over the pH range investigated, an expression, relating the observed first-order rate constant to the hydrogen-ion activity, can be derived for CI-988 and **I**:

$$k_{obs} = \left(k_0 a_H + k_{OH} K_a \frac{K_w}{a_H}\right)\left(\frac{1}{a_H + K_a}\right) \qquad (1)$$

where a_H is the hydrogen-ion activity, K_a denotes the ionization constant of the terminal carboxyl group, K_w is the ion product of water, k_0 represents the rate constant for spontaneous

degradation of the free acid, and k_{OH} is the rate constant for the specific-base catalyzed degradation of the anion.

For **II**, an additional kinetic term is needed in Eqn 1 to adequately describe the profile; a term representing specific-acid catalyzed hydrolysis of the free acid (i.e., $k_H a_H f_{HA}$ where k_H is the specific-acid catalyzed rate constant and f_{HA} is the fraction of **II** existing in the free acid form) was incorporated into Eqn 1.

For **III**, where the compound is now a base instead of an acid, a different expression, relating the observed first-order rate constant to the hydrogen-ion activity, is needed:

$$k_{obs} = \left(k'_{OH}\frac{K_a}{a_H} + k''_{OH}\right)\left(\frac{K_W}{a_H + K_a}\right) \qquad (2)$$

where k'_{OH} and k''_{OH} are the rate constants for the specific-base catalyzed degradation of the neutral and cationic species of **III**, respectively. (The k'_{OH} term is kinetically equivalent to water-catalyzed or spontaneous degradation of the neutral species.)

Table 1 shows the mechanistically important rate constants which were generated from curve fitting the experimental data to the corresponding kinetic expressions. For **II**, the K_a was set equal to the kinetically generated K_a for CI-988 to aid the curve-fitting process, since an additional kinetic term was involved. It was assumed that addition of a methylene unit to the side chain (i.e., the structural difference between **II** and CI-988) would have minimal effects on the pK_a as seen with simple alkyl carboxylic acids (March, 1985).

TABLE 1

The rate constants for the two distinct reaction pathways and the apparent ionization constants for CI-988 and its analogs at 80°C and $\mu = 0.5$ M (with NaCl) [a]

Compound	k_0 (h^{-1})	k_{OH} (M^{-1} h^{-1})	K_a
CI-988	$5.43\ (\pm 0.66)\times 10^{-2}$	$1700\ (\pm 3)$	6.30×10^{-5}
I	$5.62\ (\pm 0.05)$	$1560\ (\pm 16)$	8.19×10^{-5}
II [b]	$3.16\ (\pm 16.6)\times 10^{-3}$ [c]	$1700\ (\pm 3)$	6.30×10^{-5} [d]
III	–	$1640\ (\pm 3)$ [e]	3.38×10^{-7}

[a] The values in parentheses are the standard errors.

[b] The rate constant for specific-acid catalysis of the neutral species of **II**, k_{H^+}, is $1.08(\pm 7.01)\times 10^{-1}$ M^{-1} h^{-1}.

[c] The degradation mechanism is no longer carboxyl-assisted amide bond cleavage.

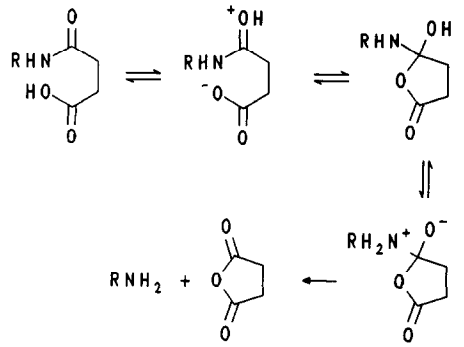
[d] This value is set equal to the K_a found for CI-988 to aid in the curve fitting, since an additional kinetic term (i.e., k_H) is required to describe the pH-rate profile.

[e] Represents hydroxide-ion catalyzed degradation of the neutral species of **III** and is denoted by k'_{OH} in Eqn 2. The comparable rate constant for the cationic species, k''_{OH}, is $4.24(\pm 0.35)\times 10^5$ M^{-1} h^{-1}.

Predominant mechanisms of degradation

For the k_0 pathway for CI-988 and **I** and for the k_H pathway for **II**, the predominant degradation reaction was consistent with intramolecular, carboxyl-facilitated, amide-bond cleavage. This was substantiated by comparison of UV spectra and co-elution on HPLC of an authentic sample of the product (i.e., **III**). For **III**, which is incapable of reacting by this pathway due to the absence of the oxobutanoic acid side chain, the k_0 value was not determined; however, the positive divergence of the experimental data from the theoretical line at pH < 5 (see Fig. 1) suggests the involvement of a pathway involving water-catalyzed or spontaneous degradation of the cationic species. (Attempts to fit the existing data with this additional kinetic term failed.) Lastly, for **II**, the k_0 pathway was inconsistent with the above mechanism due to the appearance of two different primary products; one of which appeared to be the product observed for the k_{OH} pathway.

A suitable mechanism for the k_0 pathway of CI-988 and **I** is shown in Scheme 1 (Bender et al., 1958; Kirby and Fersht, 1971) where a pre-equilibrium transfer of the carboxyl proton to the amide group is followed by ring closure to a neutral tetrahedral intermediate. An ensuing proton transfer step results in the formation of a zwitterionic tetrahedral intermediate which allows for the more facile expulsion of the amino leaving group and formation of succinic anhydride. The rate-determining step probably involves breakdown of the tetrahedral intermediate as was found for the mechanistically comparable *N*-methylmaleamic acid (Kirby and Lancaster, 1972) and *N*-phenylphthalamic acids (Hawkins, 1976; Blackburn et al., 1977).



Scheme 1. Proposed scheme for the degradation via the k_0 pathway where R represents the remainder of the CI-988 or I molecule. (The scheme depicts the reaction involving CI-988.)

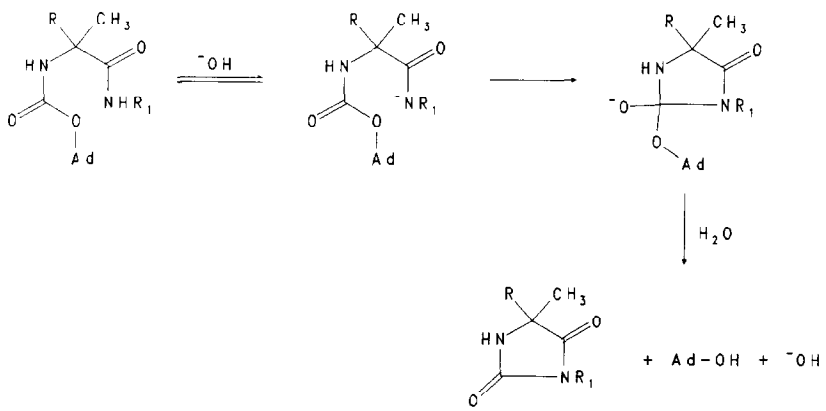
For the k_{OH} pathway, the predominant degradation reaction was consistent with intramolecular cyclization to a hydantoin product with expulsion of 2-adamantanol. This reaction was supported by the occurrence of hydantoin formation, in basic aqueous-methanolic solutions, of another CI-988 analogue (i.e., compound **III** devoid of the terminal amine) (Horwell et al., 1991). The comparable k_{OH} values (Table 1) for all of the compounds supports a common degradation mechanism. For **III**, the comparable rate constant was for the pathway involving hydroxide-ion catalyzed degradation of the neutral species. A suitable mechanism, involving specific-base catalysis and the formation and breakdown of a tetrahedral intermediate with concomitant expulsion of 2-adamantanol, is shown in Scheme 2. It 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 (Jencks, 1987).

Comparison of the reactivities of CI-988 and the analogues

The rate constants shown in Table 1 reveal that structural changes to or removal of the oxobutanoic acid side chain have a dramatic effect on the k_0 terms but have little to no effect on the k_{OH} terms (or the k'_{OH} term for **III**). This strengthens the assertion that there are two distinct reactive sites in the molecule and that the reactive site in acidic media involves the oxobutanoic acid side chain. Also, it suggests that these two sites are far enough removed so as not to influence one another to any great extent.

Comparing the rate constants for the k_0 pathway

With the mechanism proposed for the k_0 pathway (Scheme 1), changes in the structure are most likely to affect reactivity by altering the equilibrium constant for the ring-closure step rather than the rate constant for the rate-determining step (Kirby and Fersht, 1971), which is probably breakdown of the tetrahedral intermediate. This concept is an extremely important one since it is easy to lose sight of the fact that reaction steps preceding the rate-determining step contribute to the kinetics and thermodynamic quantities associated with a given reaction.



Scheme 2. Proposed scheme for degradation via the hydroxide-ion catalyzed pathway where Ad is adamantane and R and R_1 represent the remainder of the CI-988 or analogue molecule.

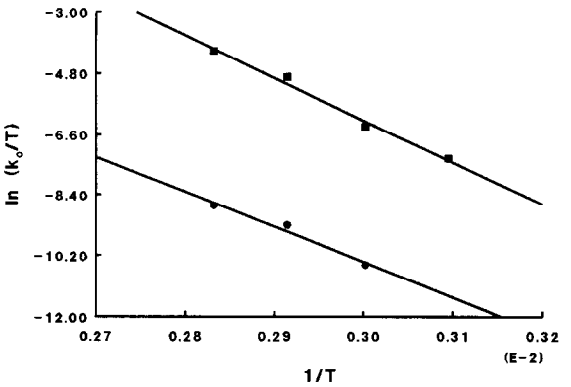


Fig. 2. The Eyring plots of the k_0 pathway for the degradation of CI-988 (●) and **I** (■) at $\mu = 0.5$ M (with NaCl).

I was found to degrade about 100-times faster than CI-988 via the k_0 pathway. The Eyring plots for **I** and CI-988 are shown in Fig. 2. A comparison of the apparent activation parameters for both compounds (Table 2) reveals that this rate enhancement observed for **I** is entropically ($\Delta S^{\ddagger}$) driven. (The enthalpies of activation, $\Delta H^{\ddagger}$, actually favor the opposite trend.) For **I**, which is more rigidly fixed in a favorable conformation for ring closure, there is a higher probability of proceeding on to the ring-closed intermediate. Similar effects of steric factors on $\Delta S^{\ddagger}$ have been seen with the degradation of 3- and 3,3′-alkyl substituted glutarate monoesters, monoesters of dicarboxylic acid which degrade via a comparable ring-closed intermediate (Bruice and Bradbury, 1968).

A comparison of the solvent-deuterium isotope effects (SIE) for the k_0 pathway for CI-988 and **I** (Table 2) shows that both pathways have comparable inverse SIE, and these values are similar to that found for the degradation of phthalamic acid (Bender, 1957). This finding is consistent with a common degradation mechanism (Scheme 1), with proton transfer steps occurring before the rate-determining step, and, hence, with the absence of general acid-base catalysis by the buffer species.

TABLE 2

Apparent activation parameters and the solvent-deuterium isotope effects for the k_0 pathway for CI-988 and I

Compound	$\Delta H^{\ddagger}$ (kcal/mol) [a]	$\Delta S^{\ddagger}$ (eu) [b]	k_H/k_D [c]
CI-988	20.6 ± 3.7	−22.1	0.8
I	24.8 ± 2.0	−1.3	0.8

[a] The error limits are standard errors.
[b] Calculated at 30°C.
[c] This is the ratio of the k_0 values determined in water to those determined in deuterium oxide. The k_0 values are 6.68×10^{-2} and 7.38 h^{-1} for CI-988 and **I**, respectively.

In contrast to **I**, **II** was found to degrade about 17-times slower than CI-988 via the k_0 pathway. As mentioned previously, the predominant mechanism of degradation was no longer carboxyl-assisted amide-bond cleavage (although this pathway predominated in the specific-acid catalyzed region of the profile). There appeared to be two other predominant mechanisms of degradation, one of which appeared to be hydantoin formation.

Since the rate constants can be thought of as proportionality constants for the associated degradation pathways and since none of the amide-cleaved product was formed over the time frame studied, the rate constant for the carboxyl-assisted amide-cleavage pathway for **II** must be significantly less than the value shown in Table 1. This finding also means that the formation of a five-membered ring (as seen with CI-988) is more energetically favorable than the formation of a six-membered ring (which would be formed with **II**). This is consistent with what has been observed for monophenyl succinates and glutarates where the succinate esters (structurally similar to CI-988) degraded between 120- and 230-times faster than the corresponding glutarate esters (structurally similar to **II**) (Bruice and Pandit, 1960; Gaetjens and Morawetz, 1960).

Since **III** is devoid of the oxobutanoic acid side chain, it is not subject to the degradation pathway involving this moiety and attributed to the other analogues.

Comparing the rate constants for the k_{OH} pathway

Alterations to the oxobutanoic acid side chain had little to no effect on the rate constants for the k_{OH} pathway (Table 1), supporting a common mechanism of degradation for all of the compounds.

Differences, seen with anionic species of CI-988, **I**, and **II**, in the positions of the negative charge associated with the terminal carboxyl group or the lack of charge seen with the neutral species of **III** had little to no effect on the rate of hydantoin formation. In contrast, the presence of a positive charge seen with the cationic species of **III** had a dramatic effect on the rate of hydantoin formation; the cation degraded about 250-times more rapidly than the neutral species via the k_{OH} pathway. The formation of the cationic species also resulted in **III** degrading more rapidly than CI-988 in the pH range of 5–9. This enhanced reactivity might be due to stabilization, by the positive charge, of negative charge build-up in the transition state of the rate-determining step and/or due to the protonated amine acting as an intramolecular general-acid catalyst.

Conclusions

The addition of a methylene unit to the oxobutanoic acid side chain of CI-988 (i.e., **II**) results in a decrease in the degradation rate via the k_0 pathway. This probably results from the formation of the ring-closed intermediate becoming less energetically favorable. As seen with many other cyclization reactions (Isaacs, 1987), formation of a five-membered ring (as occurs with CI-988 and **I**) probably represents the ideal size for this reaction. In contrast, the incorporation of a benzene moiety into the intervening carbon chain of CI-988 (i.e., **I**), results in an increase in the degradation rate via the k_0 pathway. This results because **I** is more rigidly fixed than CI-988 in a favorable conformation for ring closure, hence the reaction is entropically more favorable.

The above results were not unexpected based on a number of excellent studies found in the chemical literature; however, the results observed with **III** were unexpected. We expected **III** to be less reactive than CI-988 in the pH region where the k_0 pathway predominated (pH < 6), since it was devoid of the reactive oxobutanoic acid moiety, and we expected **III** to have comparable reactivity to CI-988 in the pH region where the k_{OH} pathway predominated (pH > 6), since this portion of the molecule involved in hydantoin formation remained intact. However, **III** is more reactive than CI-988 over the pH range of 5–9. This might be due to the formation of the cationic form of **III** where development of the positive charge might stabilize, via an electrostatic interaction or proton donation, the transition state of the rate-limiting step for hydantoin formation.

As drugs become more complex and peptide-like, where electrophilic and nucleophilic groups are placed in close proximity, the occurrence of intramolecular degradation reactions should become more prevalent. Hence, a good understanding of these types of reactions and the effects of structural changes on the rates of these reactions should aid in the selection of superior candidates, from a chemical reactivity standpoint, within a given series.

References

Bender, M.L., General acid-base catalysis in the intramolecular hydrolysis of phthalamic acid. *J. Am. Chem. Soc.*, 79 (1957) 1258–1259.

Bender, M.L., Chow, Y.L. and Chloupek, F., Intramolecular catalysis of hydrolytic reactions. II: The hydrolysis of phthalamic acid. *J. Am. Chem. Soc.*, 80 (1958) 5380–5384.

Blackburn, R.A.M., Capon, B. and McRitchie, The mechanism of hydrolysis of phthalamic and *N*-phenylphthalamic acid. *Bioorg. Chem.*, 6 (1977) 71–78.

Bruice, T.C. and Bradbury, W.C., The *gem* effect. IV: Activation parameters accompanying the increased steric requirements of 3,3' substituents in the solvolysis of mono-*p*-bromophenyl glutarates. *J. Am. Chem. Soc.*, 90 (1968) 3808–3812.

Bruice, T.C. and Pandit, U.K., The effect of geminal substitution, ring size and rotamer distribution on the intramolecular nucleophilic catalysis of the hydrolysis of monophenyl esters of dibasic acids and the solvolysis of the intermediate anhydrides. *J. Am. Chem. Soc.*, 82 (1960) 5858–5865.

Gaetjens, E. and Morawetz, H., Intramolecular carboxylate attack on ester groups. The hydrolysis of substituted phenyl acid succinates and phenyl acid glutarates. *J. Am. Chem. Soc.*, 82 (1960) 5328–5335.

Glasoe, P.K. and Long, F.A., Use of glass electrodes to measure acidities in deuterium oxide. *J. Phys. Chem.*, 64 (1960) 188–190.

Hawkins, M.D., Intramolecular catalysis. Part III. Hydrolysis of 3'- and 4'-substituted phthalanilic acids [*o*-(*N*-phenylcabamoyl)benzoic acids]. *J. Chem. Soc. Perkin II*, (1976) 642–647.

Horwell, D.C., Birchmore, B., Boden, P.R., Higginbottom, M., Ping Ho, Y., Hughes, J., Hunter, J.C. and Richardson, R.S., α-Methyl tryptophanylphenylalanines and their arylethylamine 'dipeptoid' analogues of the tetrapeptide cholecystokinin (30–33). *Eur. J. Med. Chem.,* 25 (1990) 53–60.

Horwell, D.C., Ratcliffe, G. and Roberts, E., Stabilities of tryptophanylphenethylamides to acid and alkaline conditions. *Bioorg. Med. Chem. Lett.,* 1 (1991) 169–172.

Hughes, J., Boden, P., Costall, B., Domeney, A., Kelly, E., Horwell, D.C., Hunter, J.C., Pinnock, R.D. and Woodruff, G.N., Development of a class of selective cholecystokinin type B receptor antagonists having potent anxiolytic activity. *Proc. Natl. Acad. Sci. USA*, 87 (1990) 6728–6732.

Isaacs, N.S., *Physical Organic Chemistry*, Longman, Essex, U.K., 1987, pp. 608–613.

Jencks, W.P., *Catalysis in Chemistry and Enzymology*, Dover, New York, 1987, p. 24.

Kearney, A.S., Mehta, S.C. and Radebaugh, G.W., Aqueous stability and solubility of CI-988, a novel 'dipeptoid' cholecystokinin-B receptor antagonist. *Pharm. Res.,* 9 (1992) 1095–1098.

Kirby, A.J. and Fersht, A.R., Intramolecular catalysis. *Prog. Bioorg. Chem.,* 1 (1971) 1–82.

Kirby, A.J. and Lancaster, P.W., Structure and efficiency in intramolecular and enzymic catalysis. Catalysis of amide hydrolysis by the carboxy-group of substituted maleamic acids. *J. Chem. Soc. Perkin II*, (1972) 1206–1214.

March, J., *Advanced Organic Chemistry*, 3rd Edn, Wiley, New York, 1985, p. 230.

Nadzan, A.M. and Kerwin, J.F., Cholecystokinin agonists and antagonists. *Ann. Rep. Med. Chem.,* 26 (1991) 191–200.