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Prodrugs as drug delivery systems. XXX. 4-Imidazolidinones as potential bioreversible derivatives for the α-aminoamide moiety in peptides *

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Summary

The hydrolysis kinetics of five 4-imidazolidinones derived from acetone and the dipeptides Ala–Gly, Ala–Ala, Phe–Leu, Leu–Gly and Asp–Phe methyl ester were studied to assess their suitability as prodrug forms for the α-aminoamide moiety occurring in peptides. The imidazolidinyl peptides were found to undergo a complete hydrolysis in the pH range 1–10 at 37°C and most of them showed a sigmoidal pH–rate profile with maximum rates at pH > 4. The stability of the derivatives varied widely, the following half-lives being obtained at pH 7.40 and 37°C: 0.9 h (Asp–Phe methyl ester), 3.4 h (Phe–Leu), 24.6 h (Ala–Ala), 410 h (Ala–Gly) and 530 h (Leu–Gly). The major structural factor influencing the stability appeared to be the steric properties within the C-terminal amino acid residue. The 4-imidazolidinones are much weaker bases (pK_a about 3.1) than the parent dipeptides and, as determined by partition experiments in octanol–aqueous buffer systems with the Phe–Leu derivative, they are more lipophilic than the parent compounds. It is suggested that 4-imidazolidinone formation in principle may become a useful approach to bioreversible derivatization of dipeptides or other peptides containing an α aminoamide function with the aim of solving delivery problems for peptide drugs.

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Introduction

In recent years several biologically active peptides have been discovered, including peptides consisting of only two amino acids, and most certainly, the development of peptide drugs will be a major area in drug research in the future. The application of peptides as clinically useful drugs is, however, seriously hampered due to substantial delivery problems. Peptides are readily degraded by enzymes in the gastrointestinal system and are absorbed poorly, making the oral route a poor way of administration (Wiedhaup, 1981). In fact, no orally active peptidic drugs are available in the clinic today. Furthermore, peptides suffer from metabolic lability arising from hydrolysis by plasma and tissue peptidases and even simple parenteric administration is problematic, due to the short half-lives of the peptides once they reach the bloodstream. Several peptides also suffer from systemic transport problems in that they do not readily penetrate cell membranes to reach the receptor biophase or cross the blood–brain barrier (Farmer and Ariëns, 1982; Meisenberg and Simmons, 1983). Therefore, it is now generally recognized that a need exists for approaches to obtain orally effective and metabolically stable preparations or chemical forms of biologically active peptides, e.g. various peptidic hormones, neurotransmitters and neuromodulators. Such approaches may involve the design of analogs, including the so-called non-peptidic peptidomimetics (Farmer, 1980; Zeelen, 1982), or the use of various pharmaceutical techniques including polymeric controlled release systems (Wiedhaup, 1981; Siegel and Langer, 1983). An alternative approach to solve the delivery problems may be derivatization of the bioactive peptides to produce prodrugs or transport forms possessing, with respect to delivery and metabolic stability, enhanced physicochemical properties in comparison to the parent compounds. A basic requirement for the application of this approach is the availability of appropriate types of bioreversible derivatives. In our laboratory studies have been initiated to identify and evaluate such derivatives for peptides and in the present work, 4-imidazolidinones were evaluated as a potentially useful prodrug type for the α-aminoamide moiety which is found in almost all peptides.

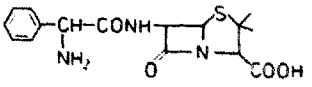
Compounds containing an α-aminoamide moiety are known to condense with aldehydes and ketones giving 4-imidazolidinones (Scheme 1). Davis and Levy (1951)

Scheme 1.

described the condensation of acetone with α-phenylglycineamide to yield 2,2-dimethyl-5-phenyl-4-imidazolidinone and later, the synthesis of a number of 4-imidazolidinones derived from various dipeptides and ketones or aldehydes was reported (Zehavi and Ben-Ishai, 1961; Yamashiro and du Vigneaud, 1968; Hruby et al., 1968; Panetta and Pesh-Imam, 1972; Ariyoshi and Sato, 1972; Hardy and Samworth, 1977). 4-Imidazolidinones were also reported to be formed by condensation of acetaldehyde with various oligopeptides (Cardinaux and Brenner, 1973), by

reaction of acetone with the tripeptide, L-propyl-L-leucyl-glycineamide (Hruby et al., 1968), and oxytocin (Yamashiro et al., 1965; Hruby et al., 1968) and by reaction of acetaldehyde with various enkephalins and β-endorphin (Summers et al., 1980; Summers and Lightman, 1981). Furthermore, 4-imidazolidinones derived from carbonyl compounds and α-aminoanilides have been reported (Katsura, 1971; Katsura et al., 1971; Nelson et al., 1973). Interestingly, the study by Nelson et al. (1973) showed the in vivo formation of a 4-imidazolidinone derivative from reaction of the key metabolite of lidocaine, N-deethyllidocaine, with acetaldehyde formed by ingestion of alcohol. Finally, the α-aminoamide side-chain in ampicillin (VI) is known to react easily with acetone yielding hetacillin (VII) (Hardcastle et al., 1966) or with other ketones or aldehydes to give the corresponding 4-imidazolidinones (Johnson and Panetta, 1965). Hetacillin is clinically used as an ampicillin prodrug as it is readily hydrolyzed to ampicillin in aqueous solution, the half-life being 15–20 min at pH 4–8 and 35 °C (Tsuji and Yamana, 1974) and about 11 min in vivo as determined after intravenous administration in man (Jusko and Lewis, 1973; Jusko et al., 1973).

In the present paper the kinetics of hydrolysis of a number of 4-imidazolidinones derived by condensation of various dipeptides with acetone (Fig. 1) is described along with data for the lipophilicity of the derivative III and its parent dipeptide to elucidate their stability and assess their suitability as prodrug forms for dipeptides.



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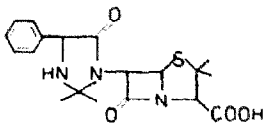


VII

Materials and Methods

Chemicals

The dipeptides studied (all of L-configuration) were purchased from AG Fluka, Switzerland or Sigma Chemicals, St. Louis, U.S.A. The 4-imidazolidinones of Ala–Gly, Ala–Ala, Leu–Gly and Phe–Leu and acetone were prepared as described by Hardy and Samworth (1977) and that of Asp–Phe methyl ester with acetone as described by Ariyoshi and Sato (1972). These 4-imidazolidinones had melting points and other physical properties in agreement with those reported in the references given.

Buffer substances and all other chemicals or solvents used were of reagent grade.

Apparatus

Visible spectral measurements were performed with a Shimadzu UV-190 spectro-

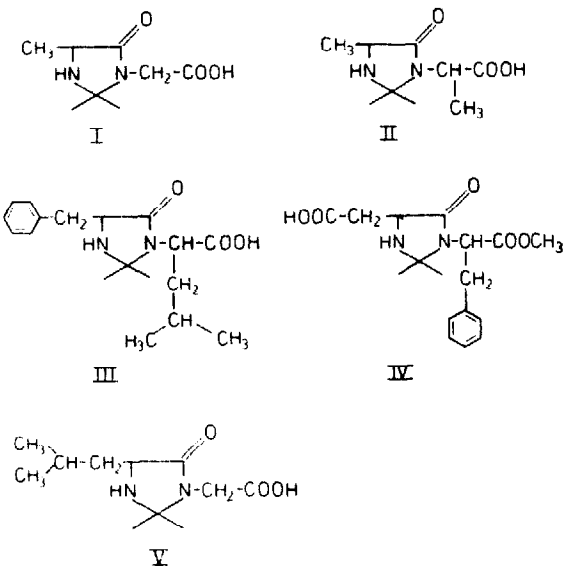


Fig. 1. Chemical structures of 4-imidazolidinones investigated in this study with the parent dipeptides (all of L-configuration) given in parenthesis: I (Ala–Gly), II (Ala–Ala), III (Phe–Leu), IV (Asp–Phe methyl ester) and V (Leu–Gly).

photometer, using 1 cm cuvettes. Readings of pH were carried out on a Radiometer Type PHM 26 meter at the temperature of study. High-performance liquid chromatography (HPLC) was done with a Spectra-Physics Model 3500B instrument equipped with a variable-wavelength UV detector (8-μl 1 cm flow cells) and a 10-μl loop injection valve. The column used, 250 × 4 mm, was packed with LiChrosorb RP-8 (7 μm particles) (E. Merck, F.R.G.).

Kinetic studies

All rate studies were performed in aqueous buffer solutions at 37.0 ± 0.2°C. The buffers used were hydrochloric acid, formate, acetate, phosphate, borate and carbonate solutions. A constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride.

The progress of hydrolysis of the various 4-imidazolidinones was generally followed by measuring the production of free peptide through analysis of their primary amino group. This primary amino group quantitation was done by using the trinitrobenzenesulphonic acid assay of Satake et al. (1960) in a modified form (cf. Bundgaard, 1976) as follows. An aliquot of 200 μl of the reaction solution containing the 4-imidazolidinone derivative of an initial concentration of about 5×10^{-4} M was added to 1000 μl of a 0.2 M borate buffer solution of pH 9.2. Thereafter, 1000 μl of a 0.2% w/v aqueous solution of 2,4,6-trinitrobenzenesulphonic acid was added and the mixture was allowed to stand at room temperature (20–25°C) for 30 min (Phe–Leu), 40 min (Ala–Ala), 45 min (Asp–Phe methyl ester), 65 min (Leu–Gly) or 75 min (Ala–Gly). Under these conditions the formation of trinitrophenylated dipeptide was found to be complete. The absorbance of the resulting orange-col-

oured solution were measured at 420 nm (1 cm cuvettes) against a blank prepared with 200 μl of water instead of the reaction solution. Pseudo-first-order rate constants were calculated from the slopes of linear plots of $\log(A_\infty - A_t)$ against time, where $A_\infty - A_t$ are the absorbance readings at 420 nm at infinity and at time t, respectively, or in cases of slow reaction rates, by the Guggenheim method for determining rates of first-order reactions. To determine the extent of reaction the absorbance readings at infinity (periods corresponding to 8–10 half-lives) were transformed to dipeptide concentration using standard curves prepared with the appropriate dipeptide.

The rate of hydrolysis of the 4-imidazolidinone derivative (III) of Phe–Leu was also determined by using a reversed-phase HPLC procedure which enabled separation and simultaneous quantitation of the imidazolidinone and Phe–Leu. The chromatographic conditions are given in the legend to Fig. 2. An accurately weighed sample of the imidazolidinone derivative (about 4 $mg \cdot ml^{-1}$) was dissolved in 0.05 M phosphate buffer pH 7.4 containing 5% of ethanol and an aliquot of 200 μl was added to 10 ml of aqueous buffer solution pre-equilibrated at 37 °C. The solution was kept at 37 °C and aliquots were removed at suitable intervals and chromatographed. Quantitation of the imidazolidinone derivative and Phe–Leu was done from measurement of the peak heights in relation to those of standards chromatographed under the same conditions. Pseudo-first-order rate constants for the hydrolysis were determined from the slopes of linear plots of the logarithm of residual imidazolidinone against time.

Some reactions were also monitored qualitatively by TLC using silica gel plates and ethyl acetate–pyridine–acetic acid–water (25 : 11 : 3 : 3 v/v) as the mobile phase. The initial 4-imidazolidinone concentration was about 3×10^{-3} M and 10 μl of the reaction solutions were chromatographed. After development the plates were sprayed with a ninhydrin reagent and heated at 100 °C.

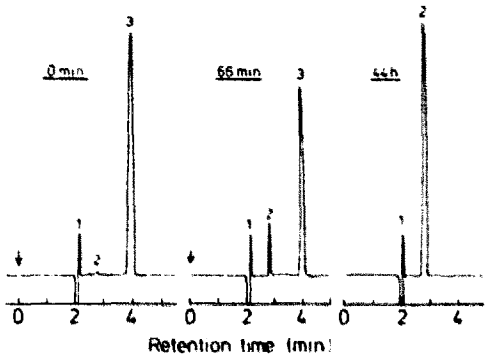


Fig. 2. High-performance liquid chromatographic traces of the degradation of the 4-imidazolidinone derivative III ($0.08\ mg \cdot ml^{-1}$) in 0.05 M phosphate buffer solution pH 7.00 at 37 °C. A 10 μl sample of the solution was chromatographed at the times indicated. Column: LiChrosorb RP-8; eluent: 0.03 M potassium dihydrogenphosphate (pH 4.5)–methanol (4 : 6 v/v); flow rate: $1.2\ ml \cdot min^{-1}$; temperature: ambient; detection: UV at 210 nm, 0.1 a.u.f.s. Peak identities: 1, solvent front; 2, Phe–Leu; 3, compound III.

Measurement of partition coefficients

The apparent partition coefficients (P) of Phe–Leu and its 4-imidazolidinone derivative III were determined in octanol–water systems at room temperature. The aqueous phase was either a 0.02 M acetate buffer solution of pH 4.00 or 0.02 M phosphate buffer solution of pH 7.40. The buffer solutions and octanol were mutually saturated at 20–25 °C before use. The compounds were dissolved in the aqueous buffer phase and the octanol–water mixtures were shaken for about 30 min to reach a distribution equilibrium. The volumes of each phase were chosen so that the solute concentration in the aqueous phase, before and after extraction, could be measured by the aforementioned HPLC method. Centrifugation was used to separate the two phases. The partition coefficients were calculated from Eqn. 1:

$$P = \left(\frac{C_i - C_w}{C_w} \right) \frac{V_w}{V_0} \quad (1)$$

where C_i and C_w represent the concentrations in the aqueous buffer phase before and after distribution, respectively; V_w represents the volume of the aqueous phase and V_0 the volume of the octanol phase.

Results and Discussion

Kinetics and mechanism of hydrolysis

The kinetics of hydrolysis of the 4-imidazolidinones I–V (N,N′-isopropylidene dipeptides) were studied in aqueous solution at 37 °C over the pH range 1–10. Under the experimental conditions used all reactions proceeded to completion (due to the slowness of reaction this was not proved for compounds I and V). The colorimetric assay for free primary amino groups showed a complete liberation of the parent dipeptides, TLC showed a complete disappearance of the imidazolidinones and, in the case of compound III, HPLC analysis revealed the formation of Phe–Leu in stoichiometric amounts. Using a TLC method, Hardy and Samworth (1977) have previously shown that various N,N′-isopropylidene dipeptides including compounds I, II, III and V are quantitatively hydrolyzed to the parent dipeptides by heating at 60 or 100 °C for 1–18 h. For compound IV, the formation of the free dipeptide methyl ester as determined by the amino group assay was observed to be followed by a slower disappearance in neutral and basic solutions. This secondary reaction of Asp–Phe methyl ester is ascribed to intramolecular aminolysis by the amino group on the methyl ester moiety, yielding a piperazine-2,5-dione. Methyl or ethyl esters of various dipeptides are known to undergo facile cyclization in weakly alkaline solutions into the corresponding piperazine-2,5-diones (Meresaar and Ågren, 1968; Purdie and Benoiton, 1973).

At constant pH and temperature, the hydrolysis displayed strict first-order kinetics and there was no evidence of any lag time in the production of free dipeptide. Fig. 3 shows some typical first-order plots for the degradation of the

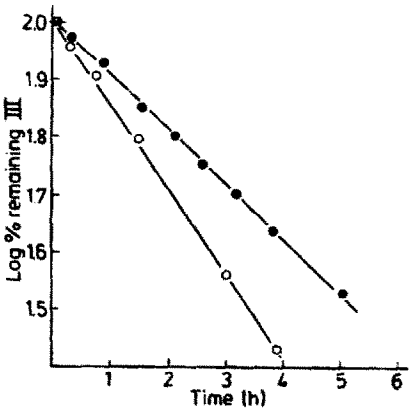


Fig. 3. First-order plots for the hydrolysis of the 4-imidazolidinone III in 0.05 M phosphate buffer solution pH 7.00 (●) and 0.05 M acetate buffer solution pH 4.00 (○) at 37 °C. The residual concentrations of compound III were determined by the HPLC method mentioned in the text.

imidazolidinone III. For this compound the rates of hydrolysis were determined using both the colorimetric assay for the parent dipeptide and the HPLC method and, as seen from the data in Fig. 4, the values of the observed pseudo-first-order rate constants (k_{obs}) derived were in favorable agreement.

The buffers used to maintain a constant pH had no measurable influence on the rates of hydrolysis of the imidazolidinones in concentrations up to 0.2 M and accordingly, the degradation is not subject to significant general acid–base catalysis.

The influence of pH on the hydrolysis rate is shown in Fig. 4, where the logarithms of the k_{obs} values are plotted against pH. As can be seen the pH–rate profiles for most compounds have a sigmoidal shape with maximum—and constant—rates at pH above 4. Compound III shows, however, a significant bell-shaped pH–rate profile with a maximum rate at about pH 4.

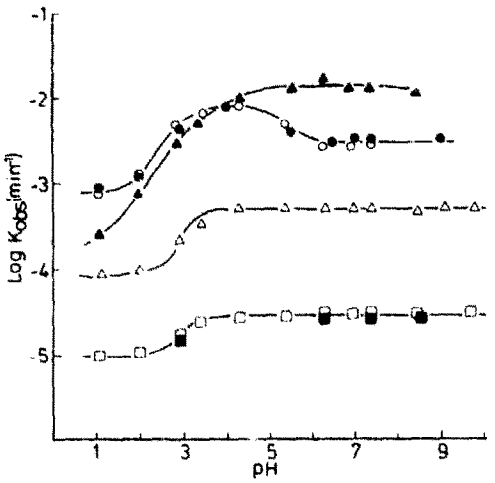
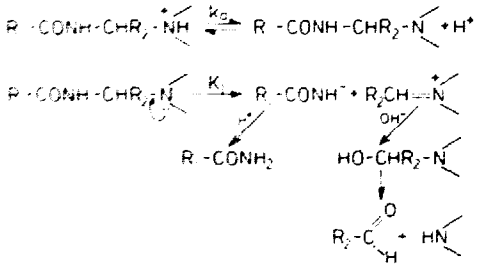


Fig. 4. pH–rate profiles for the hydrolysis of the 4-imidazolidinones I (□), II (△), III (○, ●), IV (▲) and V (■) at 37 °C ($\mu = 0.5$). For compound III the open symbols represent rate data obtained by using the colorimetric assay procedure and the filled symbols data obtained using the HPLC method.

Considering the mechanism of hydrolysis it is useful to note the structural resemblance between 4-imidazolidinones and N-Mannich bases of amides: the imidazolidinones are in fact such N-Mannich bases but with the amide and amino functions being placed in the same molecule and they may, therefore, be considered as cyclic N-Mannich bases. The hydrolysis of N-Mannich bases derived from various carboxamides, aliphatic or aromatic amines and formaldehyde (Bundgaard and Johansen, 1980a and b, 1981; Bundgaard, 1982), or isobutyraldehyde (Loudon et al., 1981) has been extensively studied and the pH–rate profiles show a sigmoidal shape similar to that for the imidazolidinones I and II. The proposed kinetic scheme for the hydrolysis of these N-Mannich bases involves spontaneous decomposition of the free Mannich bases (B) and their conjugate acids (BH^+), the expression for k_{obs} being:

$$k_{obs} = \frac{k_1 K_a}{a_H + K_a} + \frac{k_2 a_H}{a_H + K_a} \qquad (2)$$

where K_a is the apparent ionization constant of the protonated N-Mannich bases, a_H is the hydrogen ion activity, and k_1 and k_2 are the apparent first-order rate constants for the spontaneous degradation of B and BH^+, respectively. The mechanism proposed for the k_1-reaction (which predominates in weakly acidic to basic solution) involves as rate-determining step a unimolecular N–C bond cleavage with formation of an amide anion and an immonium cation. In subsequent fast steps, a solvent molecule transfers a proton to the amide anion and a hydroxide ion to the immonium ion, giving a carbinolamine, which rapidly dissociates to aldehyde and amine (Bundgaard and Johansen, 1980a and b; Loudon et al., 1981) (Scheme 2). In the k_2-reaction the most likely mechanism differs only from that for the k_1-reaction by involving expulsion of an amide enol instead of an amide anion (Loudon et al., 1981).

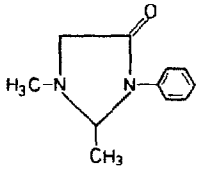


Scheme 2.

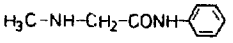
Similar mechanisms are likely to be involved in the hydrolysis of the 4-imidazolidinones. The pH–rate profiles can, at least for compounds I and II, be accounted for by the kinetic scheme expressed by Eqn. 2. In Fig. 4 the full lines for these compounds were constructed on the basis of Eqn. 2 and the following constants:
Compound I: $k_1 = 2.8 \times 10^{-5}$ min^{-1}; $k_2 = 8.9 \times 10^{-6}$ min^{-1}; $pK_a = 3.1$
Compound II: $k_1 = 4.7 \times 10^{-4}$ min^{-1}; $k_2 = 7.9 \times 10^{-5}$ min^{-1}; $pK_a = 3.1$
Due to the presence of the carboxyl group in the imidazolidinones it was not

possible to determine the pK_a values for the secondary amino groups by titrimetry. However, the values obtained from the rate data appear to be quite reasonable. By N-amidoalkylation the pK_a of amines decreases considerably, the decrease amounting to about 3 pK_a units for N-benzamidomethylation (Bundgaard and Johansen, 1980a and b). Furthermore, the pK_a value for 1,2-dimethyl-3-phenyl-4-imidazolidinone (VIII) is 3.8 whereas that of the parent α-methylaminoacetanilide (IX) is 8.1 (Katsura, 1971). Finally, Cardinaux and Brenner (1973) have reported a pK_a value of 3–4 for a 4-imidazolidinone derived from acetaldehyde and a peptide ester. The pK_a values for the amino groups in Ala–Gly and Ala–Ala are 8.1–8.2 and thus, 4-imidazolidinone formation can be seen to result in a marked depression of the amine basicity.

It should be noted that the carboxyl groups in the compounds possess pK_a values of about the same magnitude as the amino function and therefore, the sigmoidal pH–rate profiles could also be accounted for in terms of different reactivities of the carboxylic acid/carboxylate anion species. This possibility appears less likely, however, due to the N-Mannich base character of the 4-imidazolidinones.

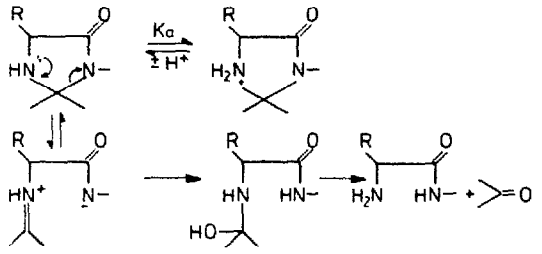


VIII



IX

The bell-shaped pH–rate profile for compound III cannot be interpreted in terms of Eqn. 2. Most certainly, this shape indicates the involvement of a kinetically significant intermediate in the reaction pathway and a change of the rate-determining step in the overall reaction with pH. The reaction mechanism proposed for the hydrolysis of the 4-imidazolidinones in their unprotonated forms is shown in Scheme 3 and is adopted from that proposed for the acyclic N-Mannich bases (Scheme 2). It



Scheme 3.

appears likely that the initial ring opening is reversible and that the Schiff base thus formed is a kinetically significant intermediate. Using an NMR spectroscopic method Durbin and Rydon (1970) have provided evidence for the existence of a Schiff base intermediate in the interconversion between ampicillin, acetone and the corresponding 4-imidazolidinone, hetacillin VII, in aqueous solution. As is the case for compound III the hydrolysis of hetacillin also shows a bell-shaped pH–rate

profile (Tsuji and Yamana, 1974) as does the imidazolidinone VIII (Katsura, 1971) as well as other 4-imidazolidinones derived from α-methylaminoacetanilide (Katsura and Sugiyama, 1971).

Lipophilicity of the imidazolidinone III and the parent dipeptide

The apparent partition coefficients (P) for the imidazolidinone III and the parent dipeptide Phe–Leu were measured using an octanol–aqueous buffer system (pH 4.0 and 7.4). The values found for log P were 1.52 (pH 4.0) and—1.02 (pH 7.4) for compound III; for Phe–Leu log P values of—0.98 (pH 4.0) and—1.25 (pH 7.4) were found. These results show that the imidazolidinone prepared from acetone is more lipophilic than the parent dipeptide, especially at pH 4 where the carboxyl group is only partly ionized. Phe–Leu occurs largely as a zwitterion at both pH values concerned. The greater lipophilicity of the imidazolidinone is also apparent from its retention time on the reversed-phase HPLC column (cf. Fig. 2).

Consideration of 4-imidazolidinones as prodrug types

The results obtained suggest that, in principle, 4-imidazolidinones may be of potential usefulness as prodrug forms for the α-aminoamide moiety occurring in e.g. dipeptides. The derivatives are hydrolyzed quantitatively to the parent compounds but with widely different rates. As can be seen from the rate data given in Table 1 the most reactive derivative (IV) exhibits a half-life of hydrolysis of less than 1 h at physiological conditions of pH and temperature whereas compounds I and V show half-lives of 400–500 h. These rates might not be expected to change much in vivo, cf. the similar stability of hetacillin in vitro and in vivo as cited above and the uneffectiveness of the decomposition rates of N-Mannich bases by human plasma (Johansen and Bundgaard, 1981). Considering the greatly different stabilities of the five 4-imidazolidinones studied the predominant structural factor influencing the reaction rate appears to be the C-terminal amino acid residue, in particular the steric properties of the α-carbon atom substituents. Thus, on going from glycine to alanine (I → II) the reactivity increases 17-fold. A further increased reactivity is seen by introducing an isobutyl group (III) or a benzyl group (IV). These results thus appear to imply that the stability of the imidazolidinyl peptides decreases with increasing steric effect within the C-terminal residue. The great reactivity of hetacillin (VII) is also in harmony with this finding. The substituents in the N-terminal amino acid, on

TABLE 1

PSEUDO-FIRST-ORDER RATE CONSTANTS (k_{obs}) AND THE CORRESPONDING HALF-LIVES ($t_{1/2}$) FOR HYDROLYSIS OF VARIOUS 4-IMIDAZOLIDINONES AT pH 7.40 AND 37 °C.

Imidazolidinone	k_{obs} (min^{-1})	$t_{1/2}$ (h)
I	2.8×10^{-5}	4.1×10^{2}
II	4.7×10^{-4}	24.6
III	3.5×10^{-3}	3.4
IV	1.3×10^{-2}	0.9
V	2.2×10^{-5}	5.3×10^{2}

the other hand, appear to have only a minor influence on the stability, cf. the almost similar stability of compounds I and V. It is obvious, however, that the present data are insufficient to delineate in detail the structural factors that may influence the stability of imidazolidinyl peptides. For the further evaluation of 4-imidazolidinones as a prodrug type for peptides it would be of special importance to establish the effect of the carbonyl component on the stability. In the related oxazolidines this effect has been shown to be substantial (Johansen and Bundgaard, 1983).

In considering 4-imidazolidinones as potential prodrug types for peptides the large decrease obtained in basicity of the reacting N-terminal amino group should also be taken into account. Such depression of amino ionization brings about an increase in the lipophilicity of the N-terminal amino acid part at physiological pH which may be of value in situations where delivery problems are due to low lipophilicity. Obviously, the type of the carbonyl component used in the imidazolidinone formation will further influence the lipophilicity by its substituents.

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