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Peptide esters as water-soluble prodrugs for hydroxyl containing agents: Chemical stability and enzymatic hydrolysis of benzyl esters of glycine, diglycine and triglycine

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Summary

The chemical stability and enzymatic hydrolysis of the mono-, di- and triglycine esters of benzyl alcohol were studied to assess the potential usefulness of peptide esters as water-soluble prodrugs for agents containing a hydroxyl group. All esters were readily hydrolyzed in 80% human plasma to give benzyl alcohol, the half-lives being 69 min for the glycine ester, 17 min for the diglycine ester and 58 min for the triglycine ester. The hydrolysis of the latter ester occurred by a two step reaction with the diglycine ester as an intermediate. The pH-rate profiles for the degradation of the esters in aqueous solution were obtained at 60°C. Maximal stability occurred at pH 3–4 at which pH the esters are highly water-soluble. The stability of the di- and triglycine esters at pH 3–5 were not markedly different and only slightly higher than that of the glycine ester. Therefore, di- and tripeptide ester prodrugs do not appear to possess significant advantages over α-amino acid esters in terms of chemical stability. From temperature-accelerated studies, the shelf-life ($t_{10\%}$) of an aqueous solution (pH 4) of the triglycine ester was thus predicted to be only 8 days at 25°C.

Introduction

Formation of α-amino acid or related short-chained aliphatic amino acid ester prodrugs has long been recognized as a useful means of increasing the aqueous solubility of drugs containing a hydroxyl group, e.g. with the aim of developing improved preparations for parenteral administration (Bundgaard, 1985). Ideally, such prodrugs should possess a high water solubility at the pH of optimum stability and sufficient stability in aqueous solution to allow long-term storage (> 2 years) of ready-to-use solutions and yet they should be converted quantitatively and rapidly in vivo to the parent drug. Considering these desirable properties of the prodrugs, the use of α-amino acid or related esters is not without problems. Although they are in general readily hydrolyzed by plasma enzymes (Bundgaard et al., 1984b; Cho and Haynes, 1985), they exhibit a poor stability in aqueous solution as exemplified with esters of metronidazole (Bundgaard et al., 1984a; Cho and Haynes, 1985), acyclovir (Colla et al., 1983), corticosteroids (Kawamura et al., 1971; Johnson

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et al., 1985) and paracetamol (Kovach et al., 1981; Jensen et al., 1991). The major reason for the high instability in aqueous solution at pH 3–5 is partly due to the strongly electron-withdrawing effect of the protonated amino group which activates the ester linkage toward hydroxide ion attack and partly to the intramolecular catalytic effect exhibited by the amino group (protonated or unprotonated) on the ester cleavage (Bruice and Benkovic, 1976; Bundgaard et al., 1984a).

As described by Anderson et al. (1985) in a study of water-soluble ester prodrugs of methylprednisolone, both these effects can be depressed somewhat by placing the amino group more distant from the ester linkage as in 6-aminocaproic acid esters. According to this approach, lengthening of the acyl part through the use of small peptides as pro-moieties may have the same depressive effect on the hydrolysis-facilitating properties of the amino group. Peptides as pro-moieties may be favorable from the point of view of safety due to their cleavage to amino acids in vivo.

In the present work, the enzymatic hydrolysis and chemical stability of the di- and triglycine esters of benzyl alcohol (**II** and **III**), used as model for a hydroxyl-containing drug, have been studied in order to evaluate the suitability of small peptide esters as water-soluble prodrugs. For comparison, the glycine ester of benzyl alcohol (**I**) was also studied.

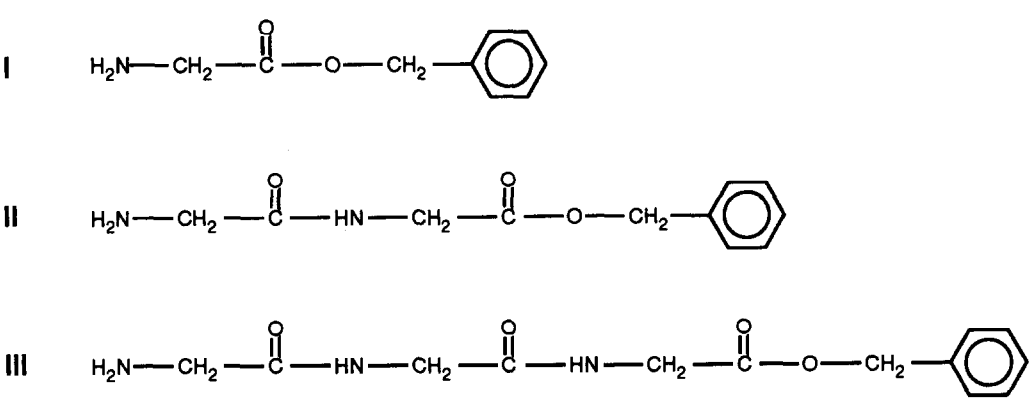
Materials and Methods

Apparatus

High-performance liquid chromatography (HPLC) was performed with a Shimadzu apparatus consisting of an LC-6A pump, an SPD-6A variable-wavelength detector and a 20 μl loop injection valve (Rheodyne). Separations were done on a deactivated reversed-phase Supelcosil LC-8-DB column (33 × 4.6 mm, 3 μm particles) equipped with a Supelguard column (purchased from Supelco Inc., U.S.A.) or a LiChrosorb RP-18 Hibar column (250 × 4.6 mm, 5 μm particles) (obtained from Merck, Darmstadt, Germany) fitted with a guard column (33 × 4.6 mm) filled with Pellicular Reversed Phase (purchased from Chrompack, The Netherlands). Readings of pH were carried out on a Radiometer PHM83 Autocal instrument at the temperature of study.

Chemicals

Glycine benzyl ester (hydrochloride), glycylglycine benzyl ester (*p*-toluenesulfonate) and glycylglycylglycine (triglycine) were purchased from Bachem AG, Bubendorf, Switzerland. Glycylglycylglycine benzyl ester was prepared from triglycine as described by Kopple et al. (1969) and isolated as the *p*-toluenesulfonate salt, m.p. 169–171°C (from ethanol); reported m.p. 168–171°C (Kopple et al., 1969).



Kinetic measurements

Hydrolysis in aqueous solutions. The hydrolysis of the esters **I–III** was studied in aqueous solutions at constant temperature (±0.2°C). The buffers used were hydrochloric acid, acetate, phosphate, borate and carbonate buffers. A constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride. The rates of hydrolysis were followed by monitoring the disappearance of the esters using a reversed-phase HPLC procedure. A mobile phase system consisting of 20% v/v acetonitrile in 0.05 M acetate buffer (pH 5.0) with 1.5 mg ml^{-1} sodium 1-heptanesulfonic acid (SHS) as an ion-paring agent was used. The system was eluted through a Supelcosil column and the effluent was monitored at 215 nm. The flow rate was adjusted between 1.0 and 1.3 ml min^{-1} to give retention times of the esters from 3 to 6 min. Benzyl alcohol and the parent peptide or amino acid appeared in the solvent front. Quantitation of the esters was done by measuring the peak heights in relation to those of standards chromatographed under the same conditions.

The reactions were initiated by adding 100 μl of a stock solution of the esters in water to 10 ml of preheated buffer solution in screw-capped test tubes, the final concentration of the esters being about 10^{-4} M. The solutions were kept in a water bath at constant temperature and at appropriate intervals, samples were taken and chromatographed immediately. For rapidly proceeding reactions (at pH > 10), the reactions were stopped by adding the samples withdrawn to a equal amount of 1 M acetate buffer (pH 4) before analysis. Pseudo-first-order rate constants for the degradation of the esters were determined from the slopes of linear plots of the logarithm of residual ester against time.

Hydrolysis in human plasma. The esters **I–III** were incubated at 37°C in human plasma diluted to 80% with 0.05 M phosphate buffer of pH 7.4. The initial concentration of the esters was 10^{-4} M. At appropriate intervals, samples of 500 μl of the plasma reaction solutions were withdrawn and added to 500 μl of a solution containing 0.1 M zinc sulfate-5% v/v perchloric acid (1 : 1 v/v) in order to deproteinize the plasma. After mixing and centrifugation for 3 min at 13000 rpm, 20 μl of the supernatant was analyzed by HPLC. A LiChrosorb column was eluted with a solvent system consisting of 10% v/v acetonitrile in 0.05 M acetate (pH 5.0) at a flow rate of 1.0 ml min^{-1}, the effluent being monitored at 215 nm. Under these conditions the retention times of the ester **I**, **II** and **III** were 11.8, 18.0 and 19.4 min, respectively, whereas benzyl alcohol eluted after 13.8 min. Quantitation of the esters was carried out by measuring the peak heights in relation to those of standards chromatographed under the same conditions.

Results and Discussion

Solubility

The benzyl esters **I–III** were readily soluble (> 10%) in water in the form of hydrochloric acid or *p*-toluenesulfonate salts.

Enzymatic hydrolysis

The susceptibility of the mono-, di- and triglycine benzyl esters to undergo enzymatic hydrolysis was studied in 80% human plasma at 37°C. Using the HPLC method described above it was possible to follow both the disappearance of the esters and the formation of hydrolysis products. The degradation of the esters exhibited strict first-order kinetics under the conditions used. The disappearance of ester **III** was found to be accompanied by the formation of the glycylglycine ester (**II**) which subsequently degraded to give benzyl alcohol. This final product of hydrolysis was formed in quantitative amounts as demonstrated by HPLC analysis of completed reaction solutions. The time courses of the various species are shown in Fig. 1 and the proposed hydrolysis reactions taking place are in Scheme 1 where k_1 and k_2 are pseudo-first-order rate constants for the depicted reactions. According to Scheme 1 the time dependences of the concentrations of **III**, **II** and benzyl alcohol (BzOH) are given by the following expressions:

$$[\mathrm{III}]_t = [\mathrm{III}]_0 e^{-k_1 t} \qquad (1)$$

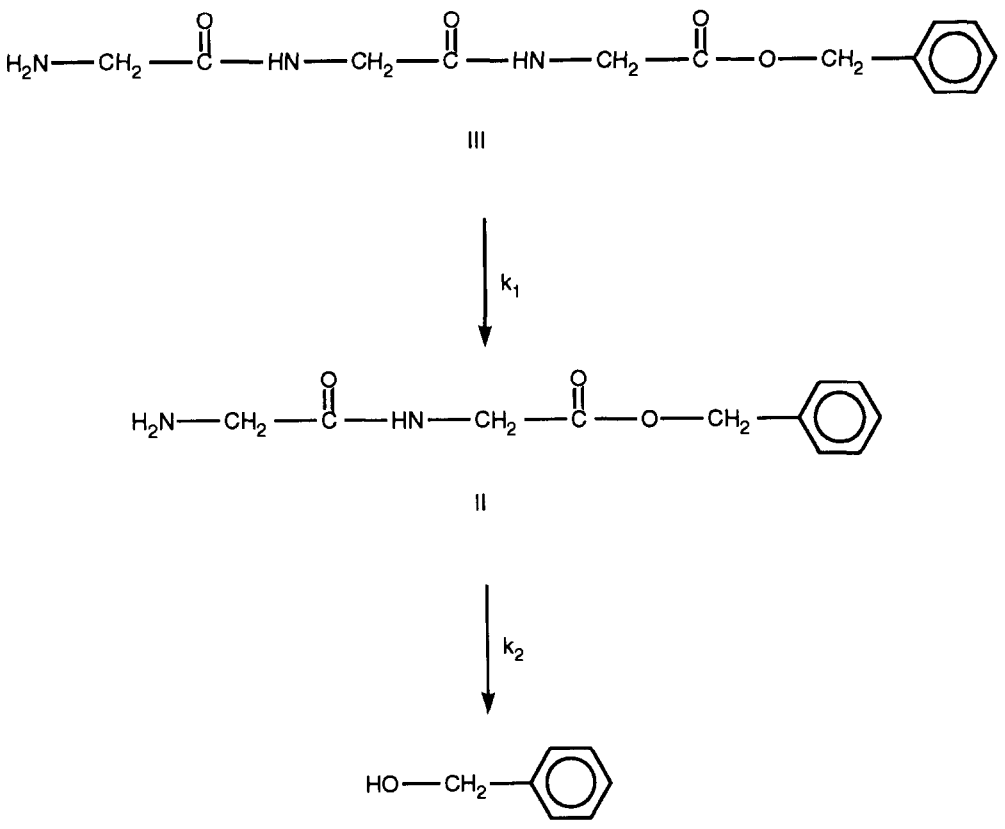
$$[II]_t = [III]_0\left[\frac{k_1}{k_2 - k_1}e^{-k_1 t} + \frac{k_1}{k_1 - k_2}e^{-k_2 t}\right] \quad (2)$$

$$[BzOH]_t = [III]_0 - ([III]_t + [II]_t) \quad (3)$$

where $[III]_0$ is the initial concentration of ester **III**. The rate constant k_1 was determined from a first-order plot of ester **III** vs time to be 0.012 min^{-1}. A value of 0.040 min^{-1} for k_2 was obtained by following the rate of hydrolysis of ester **II** separately under the same conditions (Fig. 2). Using these constants and Eqns 1–3 the solid curves in Fig. 1 were constructed. The good agreement seen between calculated and experimental data demonstrates that Scheme 1 adequately describes the kinetics of hydrolysis of the triglycine ester (**III**).

The results obtained imply that the expected direct hydrolysis of ester **III** to benzyl alcohol and triglycine in fact does not occur to any significant (< 5%) extent. Most probably, the plasma-catalyzed conversion of the triglycine ester to the diglycine ester is due to hydrolysis by an aminopeptidase in the plasma.

Neither the triglycine ester (**III**) nor the diglycine ester (**II**) produced the glycine ester (**I**) as an intermediate in their degradation pathways to benzyl alcohol. The rate constant for the degradation of ester **I** in 80% human plasma at 37°C was determined to be 0.010 min^{-1}. Therefore, the occurrence of ester **I** would readily have been detected, if it was formed, in the degradation pathway of the esters **II** and **III**. The observed half-lives for the hydrolysis of the esters in 80% human plasma solutions are listed in Table 1 along with the half-lives of hydrolysis in pure buffer solutions



Scheme 1.

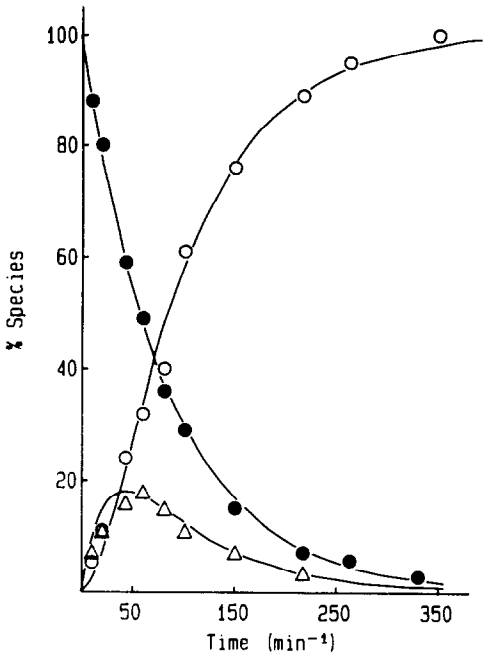


Fig. 1. Time courses for ester **III** (●), ester **II** (△) and benzyl alcohol (○) during incubation of ester **III** in 80% human plasma at 37°C.

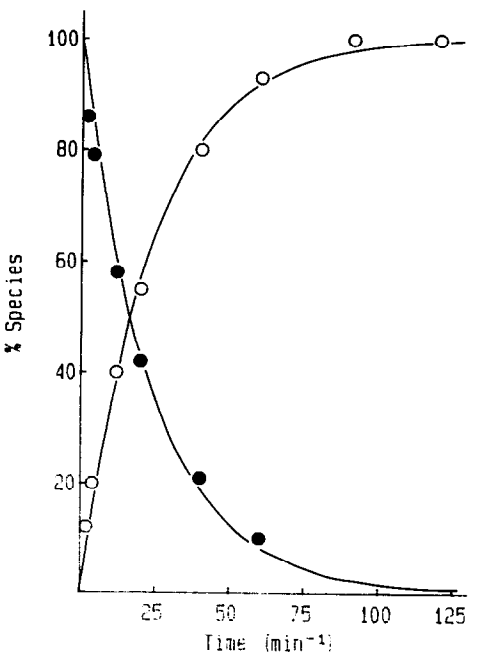


Fig. 2. Time courses for ester **II** (●) and benzyl alcohol (○) during incubation of ester **II** in 80% human plasma at 37°C.

TABLE 1

*Half-lives for the hydrolysis of esters **I–III** in 80% human plasma (pH 7.40) and 0.01 M phosphate buffer pH 7.40 at 37°C*

Ester	$t_{1/2}$ (min)	
	In human plasma	In buffer
I	69	213
II	17	40
III	58	1479

at similar pH and temperature. As appears from the data, the hydrolysis of the mono- and diglycine esters is only catalyzed to a minor extent by plasma whereas the rate of hydrolysis of the triglycine ester is markedly accelerated.

Stability in aqueous solution

The kinetics of hydrolytic breakdown of the esters **I–III** was studied in aqueous solutions at 60°C over the pH range 1.1–10.8. Under the experimental conditions used, the hydrolysis of the esters followed strict first-order kinetics over several half-lives and proceeded with the quantitative formation of benzyl alcohol as revealed by HPLC analysis. The hydrolysis of ester **II** was

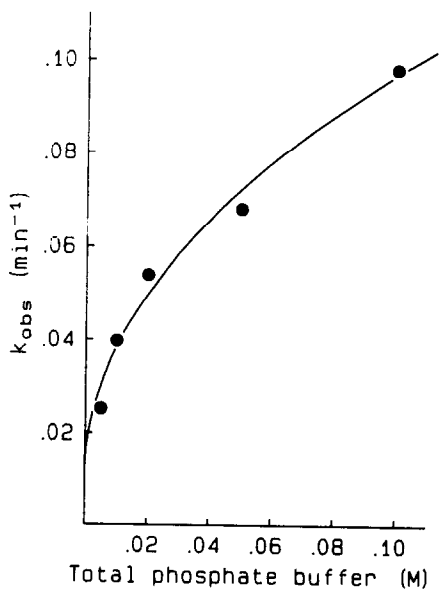


Fig. 3. The effect of phosphate buffer concentration on the observed rate constants for the degradation of ester **II** at pH 6.9 ($\mu = 0.5$, 60°C).

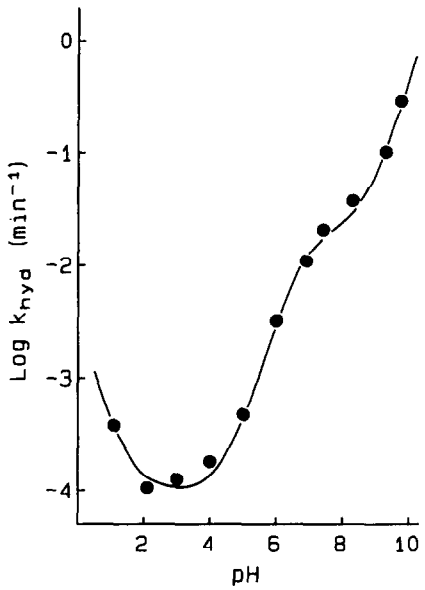


Fig. 4. The pH-rate profile for the degradation of ester **I** in aqueous solution ($\mu = 0.5$) at 60°C.

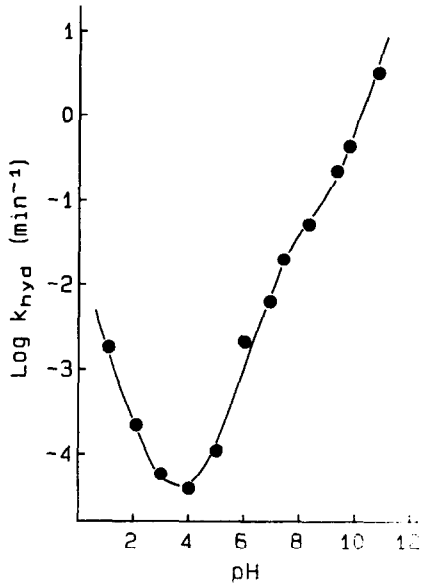


Fig. 5. The pH-rate profile for the degradation of ester **II** in aqueous solution ($\mu = 0.5$) at 60°C.

subject to marked catalysis by phosphate buffers. The catalytic effect of the buffer at pH 6.9 is shown in Fig. 3. At pH 6 and 7.4 similar non-linear phosphate buffer-catalytic effects were seen.

Variation of the concentration (0.005–0.02 M) of the acetate, borate and carbonate buffers at a given pH produced no significant change in the hydrolysis rate. For ester **I** and **III** no significant buffer catalysis was observed at the buffer concentration used (0.02 M).

The influence of pH on the rates of hydrolysis of the esters **I–III** at 60°C is shown in Figs 4–6 in which the logarithm of the buffer-independent pseudo-first-order rate constants (k_{hyd}) is plotted against pH. For esters **II** and **III** maximal stability occurs at pH about 4 whereas ester **I** is most stable at pH around 3. In the pH range studied the esters can occur in two forms with the amino function being protonated or unprotonated. The shapes of the pH-rate profiles indicate that the free base and the protonated forms of the esters undergo degradation at different rates and that the degradation can be described in terms of specific base-catalyzed reactions involving both species and a spontaneous and specific acid-catalyzed reaction of the protonated ester as depicted

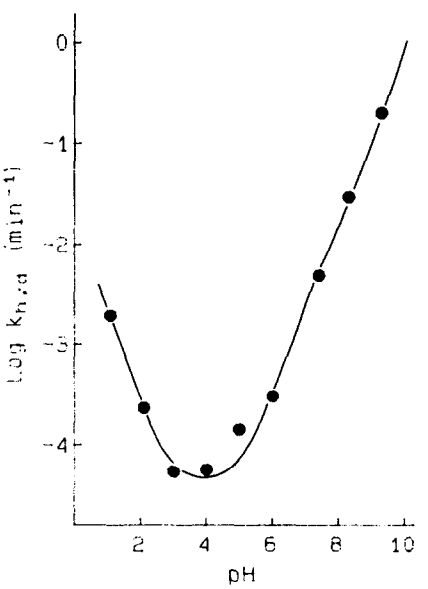
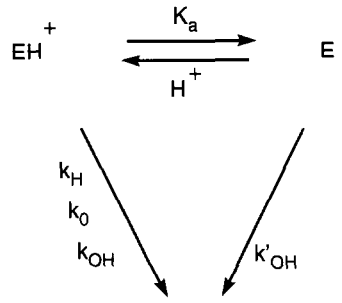


Fig. 6. The pH-rate profile for the degradation of ester **III** in aqueous solution ($\mu = 0.5$) at 60°C.



Scheme 2.

in Scheme 2 where EH^+ and E refer to the protonated and unprotonated ester forms, respectively. The following rate expression can be formulated:

$$k_{hyd} = k_H a_H \frac{a_H}{a_H + K_a} + k_0 \frac{a_H}{a_H + K_a} + k_{OH} a_{OH} \frac{a_H}{a_H + K_a} + k'_{OH} a_{OH} \frac{K_a}{a_H + K_a} \quad (4)$$

where a_H and a_{OH} refer to the hydrogen ion and hydroxide ion activities, respectively, $a_H/(a_H + K_a)$ and $K_a/(a_H + K_a)$ are the fractions of total ester in the protonated and free base forms, respectively, and K_a is the apparent ionization constant of the protonated amino group in the esters. The rate constant k_0 refers to the spontaneous or water-catalyzed hydrolysis of the protonated form of the ester, k_H is the specific acid-catalyzed rate constant for protonated ester, and k_{OH} and k'_{OH} are the second-order rate constants for the hydroxide ion-catalyzed degradation of the protonated and unprotonated ester species, respectively. The a_{OH} values were calculated from the measured pH at 60°C according to the following equation (Harned and Hamer, 1933):

$$\log a_{OH} = pH - 13.02 \quad (5)$$

The various rate and ionization constants derived from the pH-rate profiles are listed in Table 2. Using these constants the solid curves in Figs 4–6 were constructed. As will be discussed below a kinetically equivalent reaction to the k_{OH} reaction is a spontaneous reaction of unprotonated ester.

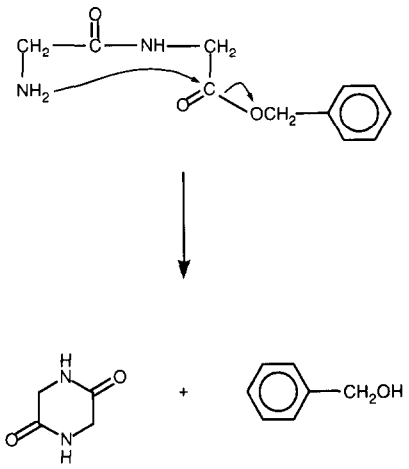
The data in Table 2 show that there is only a minor difference between the reactivity of the unprotonated forms of the esters toward hydroxide ion-catalyzed hydrolysis. This is consistent with the fact that both the steric properties within the ester moieties and the polar effects of the acyl portions of the unprotonated esters are almost the same in the three compounds. Thus, the Taft polar substituent parameter σ^* is 0.50 for $-CH_2NH_2$ and 0.56 for $-CH_2NHCOCH_3$ (Perrin et al., 1981). In its protonated form, however, the amino group is strongly electron-withdrawing ($\sigma^* = 2.2$ for $-CH_2NH_3^+$) and this may contribute to the high k_{OH} value for ester **I**. The σ^* values for the acyl portions in ester **II** and **III** are rather insensitive to protonation of the terminal amino group because of the longer distance of this group to the ester linkage. This is reflected in the similarity of the k_{OH} and k'_{OH} values for the ester **III**. For the diglycine ester **II**, on the other hand, the k_{OH} value is 15-fold larger than the k'_{OH} value and this difference cannot be accounted for in terms of polar effects. The high reactivity of the ester **II** in neutral and weakly basic solutions can rather be

TABLE 2

*Ionization constants and rate data for the degradation of the esters **I–III** in aqueous solution ($\mu = 0.5$; 60°C)*

Ester	k_H (M^{-1} min^{-1})	k_0 (min^{-1})	k_{OH} (M^{-1} min^{-1})	k'_{OH} (M^{-1} min^{-1})	pK_a [a]
I	3.5×10^{-3}	1.0×10^{-4}	3.5×10^{4}	5.0×10^{2}	6.8
II	2.1×10^{-2}	3.0×10^{-5}	1.0×10^{4}	6.8×10^{2}	7.8
III	2.3×10^{-2}	4.0×10^{-5}	3.0×10^{3}	1.0×10^{3}	7.6

[a] Kinetically determined values.



Scheme 3.

explained by an intramolecular aminolysis resulting in the formation of 2,5-piperazinedione (Scheme 3). Such reaction has previously been found to be the predominating degradation pathway for methyl and ethyl esters of diglycine and other dipeptides at pH 7–9 (Purdie and Benoiton, 1967; Meresaar and Ågren, 1968). The reaction is subject to a marked general base catalysis and the strong phosphate buffer catalytic effect seen with ester **II** may accordingly be a catalysis of 2,5-piperazinedione formation. In Eqn 4 the k_{OH} term for ester **II** should hence be replaced by a kinetically equivalent k_0' term where k_0' is a first-order rate constant for the ring closure of unprotonated dipeptide ester. Conversion of the k_{OH} value of 1.0×10^4 M^{-1} min^{-1} to k_0' gives a value of 0.060 min^{-1} for the latter.

In acidic solutions the glycine ester is about 6-fold more stable than the di- and triglycine esters. This may most probably be ascribed to greater electrostatic repulsion of hydrogen ions by the protonated amino group in ester **I** relative to esters **II** and **III** where the charged group is more remote from the reaction site. A similar trend has been reported for the acid hydrolysis of other positively charged esters (Wright, 1969).

At the pH of maximum stability of ester **III**, i.e. pH 4, the predominant degradation is water-catalyzed hydrolysis of the protonated ester species (the k_0 reaction). As seen from Table 2 the k_0 value for ester **III** is only slightly lower than that for the glycine ester. This is difficult to rationalize as one should expect ester **III** to be much more stable because of the markedly lower polar effect of its acyl moiety relative to that of the protonated glycyl group in **I**.

In order to predict the stability of the triglycine ester (**III**) in aqueous solution at normal storage temperature the rate of hydrolysis in 0.02 M acetate buffer of pH 4.0 was also determined at 70 and 80°C. By plotting the rate constants obtained according to the Arrhenius equation, an energy of activation of 8.1 kcal mol^{-1} and a log A value of 0.9 (in min^{-1}) were obtained. On the basis of these data it is possible to estimate the shelf-life (i.e. the time required to degrade 10% of the ester) at pH 4.0 and 25°C to be 8 days.

Conclusions

This study indicates that the design of a di- or tripeptide ester of a hydroxyl-containing drug is not a more valuable prodrug approach than simple α-amino acid ester formations as far as chemical stability is concerned. The di- and triglycine esters of benzyl alcohol possess only a slightly higher stability at pH 3–4 than the glycine ester which may be attributable to the electron-withdrawing effect of the peptide bond adjacent to the ester moiety. As recently described elsewhere (Bundgaard et al., 1989; Jensen et al., 1991), a far better approach to obtain both enzymatically labile and chemically stable, water-soluble prodrugs of hydroxyl-containing agents is esterification with 3- or 4-aminomethylbenzoic acid derivatives. The interesting sequential plasma-catalyzed degradation of the triglycine ester should, however, be emphasized. The ester moiety in this compound shows a very high resistance to enzymatic cleavage by plasma and is only hydrolyzed when the terminal glycine residue has been split off. Thus, the tripeptide ester behaves as a double prodrug (Bundgaard, 1989). Studies are underway to explore potential applications of this behaviour and to examine other types of tripeptide ester derivatives.

References

Anderson, B.D., Conradi, R.A. and Knuth, K.E., Strategies in the design of solution-stable, water-soluble prodrugs. I: A physical-organic approach to pro-moiety selection for 21-esters of corticosteroids. *J. Pharm. Sci.*, 74 (1985) 365–374.

Bruice, T.C. and Benkovic, S.J., *Bioorganic Mechanisms*, Vol. I, W.A. Benjamin, New York, 1966, pp. 134–145.

Bundgaard, H., Design of prodrugs: Bioreversible derivatives for various functional groups and chemical entities. In Bundgaard, H. (Ed.), *Design of Prodrugs*, Elsevier, Amsterdam, New York, 1985, pp. 1–92.

Bundgaard, H., The double prodrug concept and its applications. *Adv. Drug. Deliv. Rev.*, 3 (1989) 39–65.

Bundgaard, H., Falch, E. and Jensen, E., A novel solution-stable, water-soluble prodrug type for drugs containing a hydroxyl or an NH-acidic group. *J. Med. Chem.*, 32 (1989) 2503–2507.

Bundgaard, H., Larsen, C. and Arnold, E., Prodrugs as drug delivery systems. XXVII. Chemical stability and bioavailability of a water-soluble prodrug of metronidazole for parenteral administration. *Int. J. Pharm.*, 18 (1984a) 79–87.

Bundgaard, H., Larsen, C. and Thorbek, P., Prodrugs as drug delivery systems. XXVI. Preparation and enzymatic hydrolysis of various water-soluble amino acid esters of metronidazole. *Int. J. Pharm.*, 18 (1984b) 67–77.

Cho, M.J. and Haynes, L.C., Serum-catalyzed hydrolysis of metronidazole amino acid esters. *J. Pharm. Sci.*, 74 (1985) 883–885.

Colla, L., De Clercq, E., Busson, R. and Vanderhaeghe, H., Synthesis and antiviral activity of water-soluble esters of acyclovir (9-((2-hydroxyethoxy)methyl)-guanine). *J. Med. Chem.*, 26 (1983) 602–604.

Harned, H.S. and Hamer, W.J., The ionization constant of water in potassium chloride solutions from electromotive forces of cells without liquid junction. *J. Am. Chem. Soc.*, 55 (1933) 2194–2206.

Jensen, E., Bundgaard, H. and Falch, E., Design of a water-soluble, solution-stable and biolabile prodrug of metronidazole for parenteral administration: *N*-substituted aminomethylbenzoate esters. *Int. J. Pharm.*, 58 (1990) 143–153.

Jensen, E., Falch, E. and Bundgaard, H., Water-soluble aminoalkylbenzoate esters of phenols as prodrugs: Synthesis, enzymatic hydrolysis and chemical stability of paracetamol esters. *Acta Pharm. Nord.*, 3 (1991) 31–40.

Johnson, K., Amidon, G.L. and Pogany, S., Solution kinetics of a water-soluble hydrocortisone prodrug: Hydrocortisone-21-lysinate. *J. Pharm. Sci.*, 74 (1985) 87–89.

Kawamura, M., Yamamoto, R. and Fujisawa, S., Pharmaceutical studies on water-soluble corticosteroid derivatives. II. Stability of hydrocortisone 21-aminoalkylcarboxylates in solution. *J. Pharm. Soc. Jap.*, 91 (1971) 863–870.

Kopple, K.D., Ohnishi, M. and Go, A., Conformations of cyclic peptides. III. Cyclopentaglycyltyrosyl and related compounds. *J. Am. Chem. Soc.*, 91 (1969) 4264–4272.

Kovach, I.M., Pitman, I.H. and Higuchi, T., Amino acid esters of phenols as prodrugs: Synthesis, and stability of glycine, β-aspartic acid, and α-aspartic acid esters of p-acetamidophenol. *J. Pharm. Sci.*, 70 (1981) 881–885.

Meresaar, U. and Ågren, A., Hydrolysis of protolytic esters. VI. Alkaline hydrolysis and ring closure of glycylglycine ethyl ester. *Acta Pharm. Suec.*, 5 (1968) 85–94.

Perrin, D.D., Dempsey, B. and Serjeant, E.P., *pK_a Prediction for Organic Acids and Bases*, Chapman and Hall, London, 1981, pp. 104–142.

Purdie, J.E. and Benoiton, N.L., Piperazinedione formation from esters of dipeptides containing glycine, alanine, and sarcosine: The kinetics in aqueous solution. *J. Chem. Soc. Perkin II* (1967) 1845–1852.

Wright, M.R., Arrhenius parameters for the acid hydrolysis of esters in aqueous solution. Glycine ethyl ester, β-alanine ethyl ester, acetylcholine, and methylbetaine methyl ester. *J. Chem. Soc. (B)* (1969) 707–710.