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Prodrugs as drug delivery systems. 65. Hydrolysis of α-hydroxy- and α-acyloxy-*N*-benzoylglycine derivatives and implications for the design of prodrugs of NH-acidic compounds

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Summary

N-Hydroxymethylation and *N*-acyloxymethylation of weakly NH-acidic compounds such as carboxamides, carbamates and ureas are not useful approaches to obtain prodrug forms of such agents because of the too high stability of the N-hydroxymethyl derivatives at physiological pH and temperature. In an attempt to explore more unstable N-hydroxyalkyl derivatives various N-hydroxyalkyl compounds derived from benzamide and glyoxylic acid derivatives were prepared and their hydrolysis kinetics studied in aqueous solution in the pH range 1–12 and in human plasma solutions. All compounds degraded with the quantitative formation of benzamide and the glyoxylic acid component (the acid itself or esters and amides thereof) and they showed both specific base and acid catalysis as well as spontaneous decomposition. The derivatives were much more unstable at pH 7.4 and 37 °C than the corresponding N-hydroxymethyl derivative. Thus, whereas the half-life of decomposition of *N*-(hydroxymethyl)benzamide is 160 h under these conditions the half-lives for the glyoxylic acid derivatives are only 22 min–6.7 h, the most reactive compounds being those derived from glyoxylic acid esters and amides. These results suggest that *N*-hydroxyalkylation of amides and similar weakly NH-acidic agents using glyoxylic acid or esters or amides thereof as the aldehyde component may be a potentially useful prodrug approach. The hydrolysis kinetics of *N*-(α-acyloxyalkyl)benzamide derivatives, prepared by acylation of the benzamide-glyoxylate derivatives, was also studied. Surprisingly, these derivatives were extremely unstable in neutral aqueous solution, rendering such compounds unsuitable as prodrug forms.

Introduction

In recent years *N*-acyloxyalkylation has become a commonly used approach to obtain prodrugs of various amides, imides, hydantoins, uracils, tertiary or *N*-heterocyclic amines and other NH-acidic compounds (Bodor, 1981; Pitman, 1981; Bundgaard, 1985). The usefulness of this approach stems from the fact that by varying the acyl portion of the derivatives it is possible to control the rate of regeneration of the parent drug and to obtain prodrug derivatives with varying physicochemical properties such as water solubility and lipophilicity. Whereas the derivatives show good stability in aqueous solution in vitro similar to other esters, in general they are cleaved rapidly in vivo by virtue of enzyme-mediated hydrolysis. The regeneration of the parent drug occurs via a

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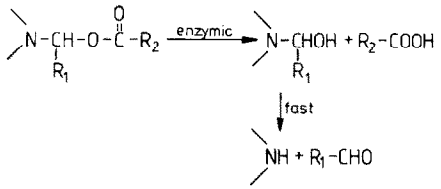
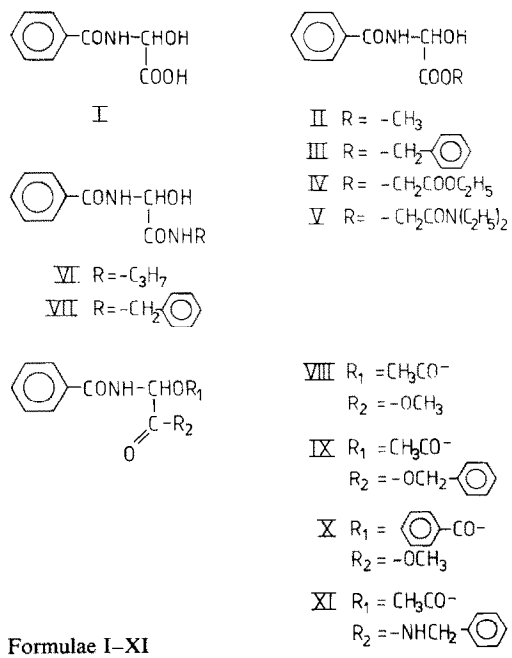


Scheme 1.

two-step reaction, enzymatic cleavage of the ester grouping followed by spontaneous decomposition of the N-hydroxyalkyl intermediate (Scheme 1).

The most commonly used acyloxyalkyl derivatives are acyloxymethyl compounds, i.e. derivatives from which formaldehyde is released from an *N*-hydroxymethyl intermediate (Bundgaard, 1985). Kinetic studies on the decomposition of a large number of *N*-hydroxymethyl derivatives (Johansen and Bundgaard, 1979; Bundgaard and Johansen, 1980) have shown, however, that *N*-acyloxymethylation is not a universally applicable approach to bioreversible derivatization of NH-acidic compounds but is limited to compounds possessing a pK_a value of less than about 10.5–11 when the requirement is to be fulfilled that the intermediate *N*-hydroxymethyl derivative should only have a transistory existence in the overall process of drug regeneration as outlined in Scheme 1. For example, *N*-hydroxymethyl derivatives of carboxamides (pK_a 14–15) are relatively stable at pH 7.4 and 37 °C, the half-lives of decomposition being more than 50–200 h (Johansen and Bundgaard, 1979).

In order to expand the usefulness of *N*-acyloxyalkylation or *N*-hydroxyalkylation as such as a means of obtaining prodrug forms of drugs containing only weakly NH-acidic moieties such as carboxamides, ureas or carbamates information is needed about the reactivity of *N*-hydroxyalkyl derivatives other than those derived from formaldehyde, i.e. *N*-hydroxymethyl derivatives. Recently, we have shown that *N*-hydroxyalkyl compounds derived from amides and the aldehydes chloral, acetaldehyde and benzaldehyde are in fact more unstable than the corresponding *N*-hydroxymethyl derivatives at physiological conditions of pH and temperature (Bundgaard and Johansen, 1984). To provide more knowledge about the reactivity of *N*-hydroxyalkyl derivatives we have now studied the behaviour of various *N*-α-hydroxyalkylamides derived from glyoxylic acid and esters and amides thereof and the present paper reports on the kinetics and mechanism of decomposition of such derivatives (I-VII) in aqueous solution at 37 °C. Furthermore, the hydrolysis kinetics of acetate and benzoate esters of some glyoxylate derivatives (VIII–XI) is reported.



Formulae I–XI

Materials and Methods

Apparatus

Ultraviolet spectral measurements were performed with a Shimadzu UV-190 spectrophotometer equipped with a thermostated cell compartment, using 1-cm quartz cuvettes. Readings of pH were carried out on a Radiometer Type PHM26 meter at the temperature of study. Melting points were taken on a capillary melting-point apparatus and are uncorrected. High-performance liquid chromatography (HPLC) was generally done with a system consisting of a Waters pump model 6000A, a variable wavelength UV-detector (Waters Type Lambda Max 480), a 20-μl loop injection valve and a Chrompack column (100 × 3 mm)

packed with CP Spher C8 (8-μm particles). In some cases a Spectra-Physics Model 3500B instrument equipped with a Lichrosorb RP-8 (7-μm particles) column (250 × 4 mm) and a 10-μl loop injection valve was used. Microanalyses were performed by G. Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, Ballerup, Denmark.

Preparation of the derivatives I–XI

α-Hydroxy-*N*-benzoylglycine (I) was prepared by reacting benzamide with glyoxylic acid monohydrate in acetone as previously described (Zoller and Ben-Ishai, 1975), m.p. 200–201°C, rep. m.p. 200.5–201.5°C.

The α-hydroxy-*N*-benzoylglycine esters II–V were prepared by alkylation of compound I with the appropriate alkyl or aralkyl halide as described for compound III. To a solution of 25 mmol (4.88 g) of compound I in 25 ml of dimethylformamide was added 25 mmol (3.5 ml) of triethylamine followed by 25 mmol (4.27 g) of benzylbromide. The solution was stirred at room temperature overnight, poured into water (150 ml) and then extracted with ethyl acetate (2 × 150 ml). The combined extracts were washed with water, 2% sodium hydrogen carbonate and water. After drying over anhydrous sodium sulphate, the ethyl acetate was removed under reduced pressure to give compound III. It was recrystallized from aqueous ethanol, yielding 5.1 g, m.p. 126–127°C, rep. m.p. 125–126°C (Chemiakine et al., 1959). Compound II (m.p. 115–116°C, from acetone–water) and compound IV (m.p. 111–112°C, from ethanol–water) had melting points in agreement with those reported for the compounds prepared by other methods (Chemiakine et al., 1959). Compound V which has not been described before showed a melting point of 81–82°C (from ethanol-ether-petroleum ether) and a microanalysis (C, H, N) within ±0.4% of the calculated values.

The α-hydroxy-*N*-benzoylglycine amides VI and VII were prepared by aminolysis of the ester IV. Thus, to a solution of 7.8 mmol (2.0 g) of compound IV in 15 ml of ethyl acetate (heated to 40°C to affect solution) was added 10 mmol of *n*-propylamine or benzylamine. After a few minutes the products VI and VII began to precipitate. The mixtures were kept for 2 h at room temperature and then cooled to 4°C and filtered, m.p. 133–135°C (VI, from ethanol) and 156–157°C (VII, from ethanol), rep. m.p. 152–153°C (Chemiakine et al., 1959). Compound VI showed a satisfactory microanalysis (C, H, N).

The α-acyloxy-*N*-benzoylglycine derivatives VIII–XI were prepared by acylating the parent α-hydroxy-*N*-benzoylglycine derivatives according to the following procedure described for VIII. A stirred suspension of compound II (0.42 g, 2 mmol) in acetic anhydride (10 ml) was cooled to approximately 0°C and pyridine (6 ml) was added. The reaction mixture was stirred for 3 h at 0°C and then kept overnight at 4°C. The mixture was concentrated in vacuo and the residue was dissolved in ethyl acetate and washed with 3 M hydrochloric acid, 5% sodium hydrogen carbonate and water. The ethyl acetate solution was dried over anhydrous sodium sulphate and evaporated under reduced pressure to leave a residue which crystallized from ether-petroleum ether to give 300 mg of compound VIII, m.p. 83–84°C. The melting points for the other esters were: 104–105°C (IX, from ether–petroleum ether), 109–110°C (X, from ether–petroleum ether) and 158–159°C (XI, from ethyl acetate–petroleum ether). The benzoate ester X was prepared by reacting a solution of compound II (3 mmol) in 5 ml pyridine with benzoyl chloride (8 mmol) at 0°C for 4 h and was isolated as described above. All derivatives showed a microanalysis (C, H, N) within ± 0.4% of the calculated values.

Kinetic measurements

All rate studies were performed in aqueous buffer solutions at 37.0 ± 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 progress of the reactions was followed either by direct UV-spectrophotometry or by HPLC. In the former method the reactions were performed in 2.5 ml aliquot portions of buffer solutions in a thermostated quartz cuvette and were initiated by adding 25 μl of stock solutions

of the derivatives in acetonitrile to give a final concentration of $0.5–2 \times 10^{-4}$ M. The rate of hydrolysis of the compounds was followed by monitoring the decrease in absorbance at 230 nm or 240 nm. Pseudo-first-order rate constants were determined from the slopes of linear plots of log $(A_t - A_\infty)$ vs time, where A_t and A_∞ are the absorbance readings at time t and infinity, respectively.

In most cases the rates of degradation were followed by using reversed-phase HPLC procedures. Mobile phase systems of 35–62% v/v methanol in 0.01 M acetate buffer of pH 5.0 were generally used, the concentration of methanol being adjusted for each compound to give an appropriate retention time (2–5 min). In the case of compound I a mobile phase consisting of methanol–0.01 M phosphate buffer pH 2.0 (2 : 3 v/v) was used. The solvent systems used allowed quantitation of the parent amides formed upon hydrolysis simultaneously with quantitation of the starting material. The column effluent was monitored at 230 or 240 nm. Quantitation of the compounds 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 compounds in ethanol or acetonitrile to 10 ml of preheated buffer solution in screw-capped test tubes, the final concentration of the compounds being about 10^{-4} M. The solutions were kept in a water bath at 37 °C and at appropriate intervals samples were taken and chromatographed. Pseudo-first-order rate constants for the degradation were determined from the slopes of linear plots of the logarithm of residual derivative against time.

In case of the hydrolysis studies performed in human plasma diluted to 80% with 0.05 M phosphate buffer of pH 7.40, samples of 250 µl were withdrawn at appropriate intervals and added to 1000 µl of ethanol in order to deproteinize the plasma. After immediate mixing and centrifugation for 2–3 min, 20 µl of the clear supernatant was analyzed by HPLC as described above.

Results and Discussion

Kinetics of hydrolysis of compounds I–VII

The kinetics of breakdown of the α-hydroxy-*N*-benzoylglycine derivatives (I–VII) was studied in aqueous solution at 37 °C over a wide range of pH. At constant pH and temperature the reactions displayed strict first-order kinetics over several half-lives (cf. Fig. 1) and all reactions proceeded to completion. As revealed by HPLC the parent amide was formed in stoichiometric amounts in all cases. An example is shown in Fig. 2. In some cases (basic solutions) the rate of a given reaction was determined using both the direct UV-spectrophotometric method and the HPLC method and the values of the rate constants obtained therefrom agreed within ± 3%.

The rates of decomposition were found to be independent of buffer concentration from 0.02 to 0.05 M at constant ionic strength. Thus, no significant general acid–base catalysis appears to be involved which is also the case for the hydrolysis of *N*-hydroxymethyl derivatives (Johansen and Bundgaard, 1979) and other *N*-hydroxyalkyl compounds (Bundgaard and Johansen, 1984).

The influence of pH on the hydrolysis rate is shown in Figs. 3 and 4, where the logarithms of the observed pseudo-first-order rate constants (k_{obs}) are plotted against pH. The pH-rate profiles for most of the derivatives are U-shaped, indicat-

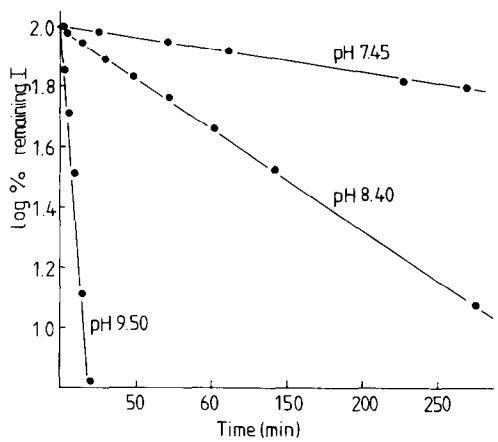


Fig. 1. First-order plots for the degradation of compound I in aqueous solution at 37 °C.

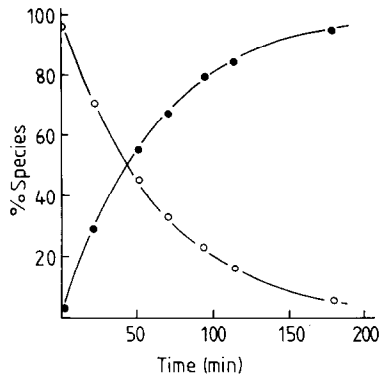


Fig. 2. Time-courses for compound II (○) and benzamide (●) in the degradation of compound II at pH 7.40 (at 37 °C).

ing the occurrence of specific acid and base catalysis as well as a spontaneous or water-catalyzed reaction according to the following rate expression:

$$k_{obs} = k_0 + k_H a_H + k_{OH} a_{OH} \quad (1)$$

where a_H and a_{OH} refer to the hydrogen ion and hydroxide ion activity, respectively. The latter was calculated from the measured pH at 37 °C according to the following equation (Harned and Hamer, 1933):

$$\log a_{OH} = \text{pH} - 13.62 \quad (2)$$

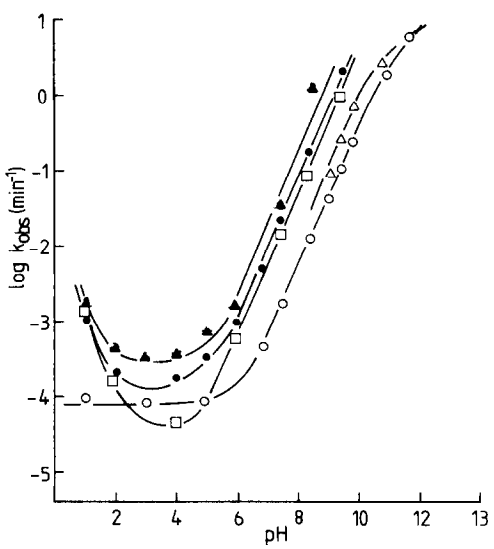


Fig. 3. The pH-rate profiles for the degradation of compound I (○), compound II (□), compound III (△), compound IV (▲) and compound V (●) in aqueous solution ($\mu = 0.5$) at 37 °C.

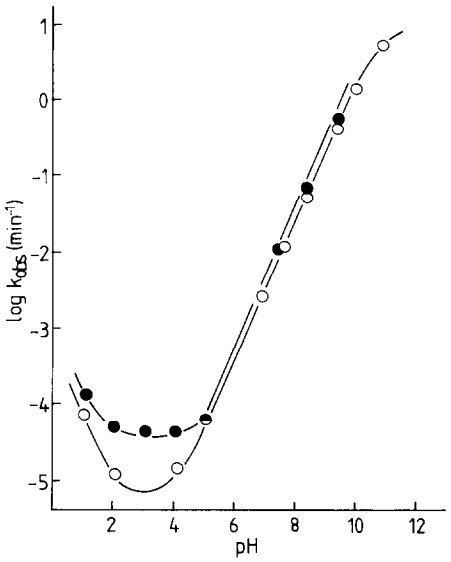


Fig. 4. The pH–rate profiles for the degradation of compound VI (●) and compound VII (○) in aqueous solution ($\mu = 0.5$) at 37 °C.

Values of the second-order rate constants for the specific acid (k_H) and specific base (k_{OH}) catalyzed hydrolysis were determined from the straight line portions of the pH-rate profiles at low and high pH values, respectively, whereas the value of the first-order rate constant for spontaneous hydrolysis (k_0) was obtained on the basis of Eqn. 1. The values of the rate constants derived are listed in Table 1. In Figs. 3 and 4 the solid curves or

TABLE 1

Rate data for the hydrolysis of various α-hydroxy-N-benzoylglycine derivatives in aqueous solution at 37 °C and $\mu = 0.5$

Compound	k_H (M^{-1} min^{-1})	k_0 (min^{-1})	k_{OH} (M^{-1} min^{-1})
I	–	8.5×10^{-5}	1.8×10^{3}
II	1.5×10^{-2}	4.4×10^{-5}	1.9×10^{4}
III	n.d.	n.d.	3.6×10^{3}
IV	1.7×10^{-2}	4.0×10^{-4}	6.0×10^{4}
V	8.6×10^{-3}	1.8×10^{-4}	2.6×10^{4}
VI	1.2×10^{-3}	4.0×10^{-5}	1.2×10^{4}
VII	8.9×10^{-4}	5.0×10^{-6}	7.6×10^{3}

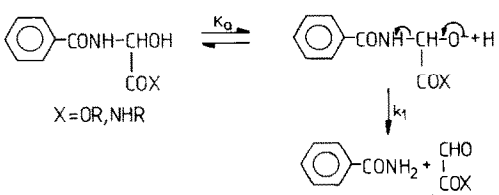
lines drawn were constructed from these constants and Eqn. 1.

In contrast to the ester and amide derivatives the free acid derivative I exhibits no specific acid-catalyzed hydrolysis at pH values greater than 1. As the pH-rate profile for the compound shows no break around pH values corresponding to the pK_a for the carboxylic acid group of the compound (3.1 at 20–25 °C) (Ravdel et al., 1968) there is apparently no significant difference in the reactivity of the ionized and unionized species.

Eqn. 1 is only valid up to pH about 10. As can be seen from Figs. 3 and 4 the rate of decomposition levels off with increasing pH at pH > about 10. A similar leveling-off of the decomposition rate at high pH has been observed for *N*-(hydroxymethyl)benzamide (Johansen and Bundgaard, 1979) and *N*-(α-hydroxy-2,2,2-trichloroethyl)-benzamide (Bundgaard and Johansen, 1984) and is consistent with a reaction mechanism involving a stepwise pathway with anionic *N*-hydroxyalkyl amide as an intermediate undergoing rate-determining N–C bond cleavage (Scheme 2) (Ugelstad and de Jonge, 1957; Johansen and Bundgaard, 1979). According to this mechanism the rate law for the reactions occurring in neutral and alkaline solutions should be written as:

$$k_{obs} = k_1 \frac{K_a}{a_H + K_a} \qquad (3)$$

where K_a is the ionization constant for the *N*-hydroxy-alkyl derivative and k_1 is a first-order rate constant for cleavage of the ionized *N*-hydroxyalkyl compound. Because of the rapid reactions taking place at pH > 11 sufficient rate data to test the validity of Eqn. 3 could not be obtained. However, the pH–rate profiles for the compounds I, III and VII indicate that the pK_a value for the



Scheme 2.

TABLE 2

Half-lives of decomposition of various α-hydroxy-N-benzoylglycine derivatives and N-(hydroxymethyl)benzamide in aqueous solution at 37 °C and μ = 0.5

Compound	Half-life (h)	
	pH 2.0	pH 7.4
N-hydroxymethyl-benzamide	n.d.	160
I	136	6.7
II	70	0.78
III	n.d.	4.5
IV	24	0.36
V	61	0.76
VI	241	1.1
VII	918	1.0

hydroxyl group in the derivatives is in the range 11–12. The pK_a of *N*-(hydroxymethyl)benzamide has been determined to be 13.1 at 37 °C (Johansen and Bundgaard, 1979) and it is to be expected that the pK_a values of the compounds I–VII are somewhat lower due to the electron-withdrawing carboxy substituents in these derivatives.

Inspection of the rate data in Table 2 shows that the glyoxylic acid derivatives I-VII are much more reactive than the corresponding *N*-(hydroxymethyl)benzamide derivative. Thus, whereas the latter is decomposed with a half-life of 160 h at pH 7.4 and 37 °C, the derivatives of benzamide with glyoxylic acid and methylglyoxylate possess half-lives of 6.7 h (I) and 47 min (II). A similar trend has been observed for glyoxylic acid derivatives of salicylamide and nicotinamide, the half-lives being 2.4 and 1.4 h, respectively, at pH 7.4 and 37 °C which should be compared with 17 and 37 h for the *N*-hydroxymethyl derivatives of salicylamide and nicotinamide, respectively (to be reported elsewhere). As previously discussed (Bundgaard and Johansen, 1984) this difference in reactivity may largely be due to steric effects within the α-substituents although polar effects may also play a role.

Recently, Ross et al. (1983) have quoted a half-life of hydrolysis of *N*-(hydroxymethyl)benzamide of only 21.1 h at pH 7.4 and 37 °C. This value is much lower than the value (160 h) found by us but it appears to be incorrect. When we

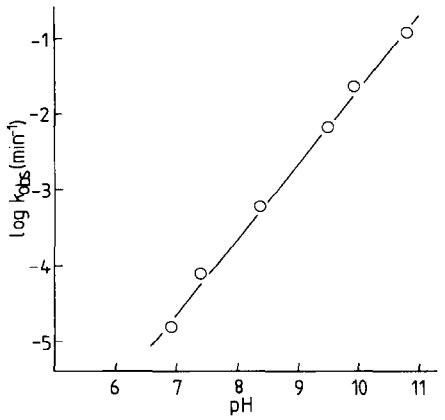


Fig. 5. The influence of pH on the rate of decomposition of *N*-(hydroxymethyl)benzamide in aqueous solution ($\mu = 0.5$) at 37 °C.

followed the hydrolysis of *N*-(hydroxymethyl)benzamide by a specific HPLC method (LiChrosorb RP-8 column, mobile phase: methanol–0.01 M phosphate buffer of pH 3.5 (35 : 65 v/v)) rate constants were obtained which agreed well with those obtained by determining the amount of formaldehyde produced during the hydrolysis (Johansen and Bundgaard, 1979). Furthermore, the rate data obtained at pH 7.4 fit well to those obtained at other pH values as seen from the pH-rate profile in Fig. 5. The slope of the line in Fig. 5 is 1.0 which is in accordance with Eqn. 3 since $a_H \gg K_a$ in the range investigated. Under such conditions Eqn. 3 takes the form of:

$$k_{obs} = k_1 a_{OH} K_a / K_w \quad (4)$$

where K_w is the ionization constant of water.

Hydrolysis in plasma solutions

The rates of decomposition of compounds I, II, V and VI were determined in 80% human plasma (pH 7.4) and 37 °C. The carboxylic acid I was slightly more labile in plasma and buffer solutions (Table 3). This lack of pronounced enzymatic catalysis is expected on the basis of the suggested reaction mechanism and had also been observed previously for other *N*-hydroxyalkyl amides (Johansen and Bundgaard, 1981; Bundgaard and Johansen, 1984).

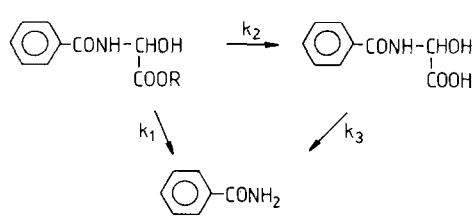
TABLE 3

Half-lives of decomposition of various α-hydroxy-N-benzoylglycine derivatives in 80% human plasma (pH 7.4) and buffer (pH 7.4) solutions at 37 °C

Compound	Half-life (min)	
	Buffer (pH 7.4)	80% Plasma
I	402	234
II	47	35 [a]
V	46	5 [a]
VI	66	72

[a] Half-life for the overall loss of the compound.

The ester derivatives II and V showed a plasma-catalyzed hydrolysis of the ester moiety occurring simultaneously with the non-enzymatic cleavage to give benzamide (Scheme 3). Fig. 6 shows the time-courses for the methyl ester II, the acid I and benzamide during the degradation of compound II in 80% human plasma. As can be seen the acid I is formed to a significant extent (69%). Following its formation it is degraded to benzamide which is produced in a quantitative yield. The pseudo-first-order rate constants depicted in Scheme 3 had the following values for compound I: $k_1 = 0.0062$ min^{-1}; $k_2 = 0.0138$ min^{-1} and $k_3 = 0.0030$ min^{-1}. In the case of the glycolamide ester V the ester cleavage is much more pronounced. The half-life of compound V was only 5 min, the reaction being due to ester hydrolysis since the acid I was formed in almost 100% yield. The observed much greater susceptibility of the *N*,*N*-disubstituted glycolamide ester V to undergo plasma-catalyzed hydrolysis relative to the corresponding methyl ester (II) is in accordance with the results obtained for such esters of a



Scheme 3.

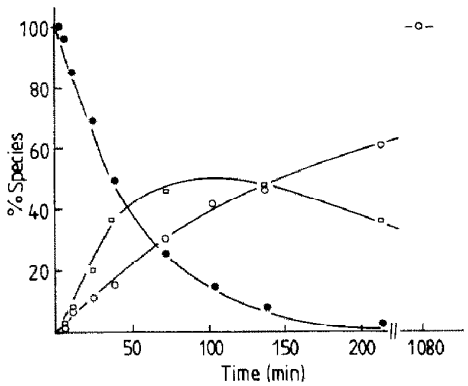


Fig. 6. Time-courses for compound II (●), compound I (□) and benzamide (○) in the degradation of compound II in 80% human plasma solutions at pH 7.4. The various compounds were determined by reversed-phase HPLC.

number of other carboxylic acids (Bundgaard and Nielsen, 1987).

Hydrolysis of the ester derivatives VIII–XI

By esterification of the hydroxy group in *N*-hydroxyalkyl derivatives chemically stable derivatives are normally obtained as mentioned in the introduction. It was, therefore, surprising to find that the ester derivatives VIII–XI decomposed extremely rapidly in weakly acidic and neutral aqueous solution. In fact, the compounds showed a higher instability than their parent α-hydroxy-*N*-benzoylglycine derivatives at all pH values.

TABLE 4

Rate data for the hydrolysis of various α-acyloxy-N-benzoyl glycine derivatives in aqueous solution at 37 °C and μ = 0.5

Compound	k_H (M^{-1} min^{-1})	k_0 (min^{-1})	k_{OH} (M^{-1} min^{-1})
VIII	6.1×10^{-2}	1.3×10^{-3}	2.9×10^{7}
IX	6.8×10^{-2}	3.1×10^{-4}	2.4×10^{7}
X	3.5×10^{-2}	1.5×10^{-3}	8.7×10^{7}
XI	5.9×10^{-1}	1.9×10^{-3}	3.1×10^{7}

The pH–rate profiles obtained for the hydrolysis of the compounds are shown in Fig. 7. The rate of hydrolysis can be described by the rate expression of Eqn. 1 and in Table 4 values of the various rate constants are listed. From the k_{OH} values the half-lives of hydrolysis at pH 7.4 and 37 °C were estimated to be 1–3 s. At pH around 2.5 the derivatives showed half-lives of 7–37 h.

The decomposition of these derivatives proceeded with the quantitative formation of the corresponding α-hydroxy derivative which subsequently hydrolyzed more slowly to give benzamide and glyoxylate as depicted in Scheme 4. This was shown by HPLC using solvent systems affording separation of the various compounds. An example of a product analysis is shown in Fig. 8.

Considering the mechanism involved in the

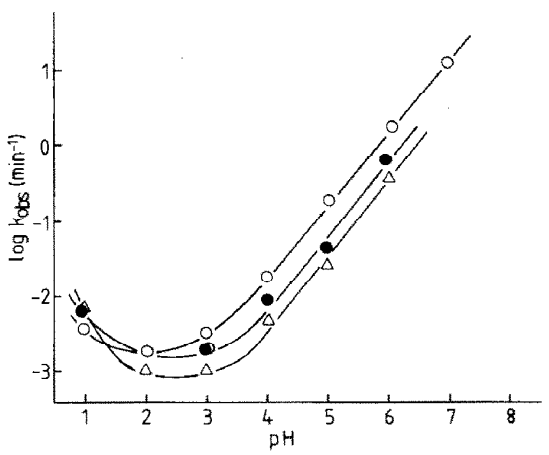


Fig. 7. The pH-rate profiles for the hydrolysis (deacylation) of compound X (○), compound VIII (●) and compound IX (△) in aqueous solution ($\mu = 0.5$) at 37 °C.

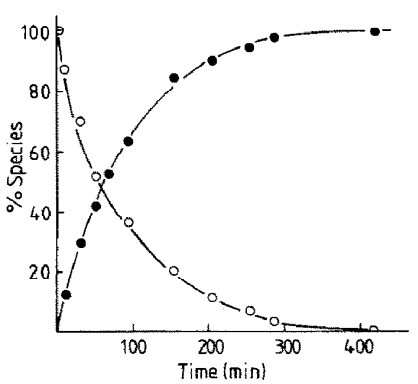
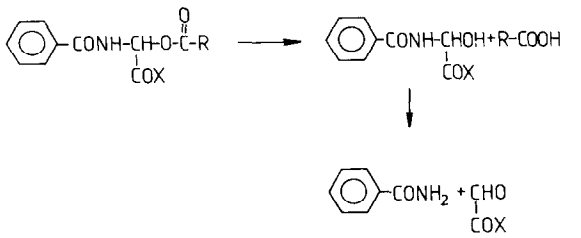


Fig. 8. Time-courses for compound VIII (○) and compound II (●) in the degradation of compound VIII in 0.02 M acetate buffer solution (pH 4.00) at 37 °C.

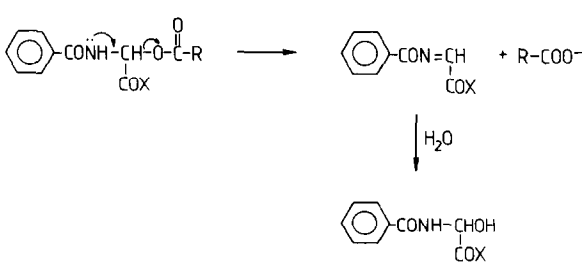


Scheme 4.

facile hydrolysis of the α-acyloxyglycine derivatives a process resulting in the formation of a transient *N*-acylimine intermediate (Scheme 5) appears most likely. The rate-determining step involves elimination of carboxylate ion to give an *N*-acylimine intermediate which in a subsequent fast step undergoes attack by a water molecule, giving the α-hydroxyglycine compound. Such a mechanism has previously been suggested in the reaction of the N-acyl-α-acetoxyamino acid derivatives with various nucleophiles including alcohols, thiols, amines and nucleic acid bases (Olsen and Kolar, 1975; Ozaki et al., 1979; Nishitani et al., 1979). Further support for the elimination mechanism is provided by comparing the k_{OH} value for compound X with the k_{OH} values for the hydrolysis of various simple benzoate esters at the same reaction conditions (37 °C, $\mu = 0.5$). For such esters the following linear free energy relationship has been established (Nielsen and Bundgaard, unpublished):

$$\log k_{OH} = 0.54\sigma^* + 0.74 \qquad (5)$$

(k_{OH} in M^{-1} min^{-1} at 37 °C) where σ^* is the Taft polar substituent parameter. In Eqn. 5 σ^* refers to R in RCH_2OH for the alcohols. Considering compound X the σ^* values for the substituents $-COOCH_3$ and $-NHCOC_6H_5$ are 2.00 and 1.68, respectively (Perrin et al., 1981). Substituting the sum of these values in Eqn. 5 affords a k_{OH} value of 533 M^{-1} min^{-1} which can be seen to be much lower than the value (8.7×10^7 M^{-1} min^{-1}) actually determined. This difference between the estimated and experimental value represents a minimum value since steric effects within the alcohol part of the ester X have not been taken into account.



Scheme 5.

Conclusions

The results obtained suggest that *N*-hydroxyalkylation of carboxamides using glyoxylic acid or esters or amides thereof as the aldehyde component may be a potentially useful approach to obtain prodrug derivatives of such weakly NH-acidic compounds. In contrast to the corresponding *N*-hydroxymethyl derivatives the glyoxylate adducts are readily hydrolyzed at physiological conditions of pH and temperature. This study has only concerned carboxamides but based on previous studies (Johansen and Bundgaard, 1979; Bundgaard and Johansen, 1980) a similar facile hydrolysis of glyoxylate adducts of other weakly NH-acidic compounds such as urea derivatives and carbamates is expected. Such derivatives are readily available by reacting the NH-acidic compound with glyoxylic acid or esters thereof (e.g. Valentine and Wolfe, 1961; Matthies, 1970; Zoller and Ben-Ishai, 1975; Ben-Ishai et al., 1977a and b; Schouteeten et al., 1978; Edwards, 1980; Nader et al., 1981; Tschaen et al. 1984).

Derivatization of weakly acidic amides and similar compounds with glyoxylic acid may be useful in efforts to e.g. improve the solubility or liphophilicity characteristics. As has been shown above the carboxy group in the derivatives is readily amenable to esterification or it can be transformed to an amide, affording ample possibilities to control the solubility and lipophilicity characteristics of the prodrug derivatives and still maintain an appropriate rate of hydrolysis at physiological pH. By selecting an amide or a sterically hindered ester plasma-catalyzed hydrolysis to give the more stable glyoxylic acid deriva-

tive can be avoided or depressed. A drawback of such derivatives may be their limited stability in aqueous solution which precludes the formulation of ready-to-use solutions. As has been demonstrated in this work it is not possible to stabilize the glyoxylate derivatives by esterification of the hydroxyl group, the ester derivatives being even more unstable than the parent *N*-hydroxyalkyl compounds.

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