International Journal of Pharmaceutics, 29 (1986) 9–18
Elsevier

IJP 00960

Prodrugs as drug delivery systems. Part 42. 2-Hydroxymethylbenzamides and 2-acyloxymethylbenzamides as potential prodrug forms for amines *

Niels Mørk Nielsen and Hans Bundgaard

The Royal Danish School of Pharmacy, Department of Pharmaceutical Chemistry AD, DK-2100 Copenhagen (Denmark)

(Received July 30th, 1985)
(Accepted October 13th, 1985)

Key words: 2-hydroxymethylbenzamides – 2-acyloxymethylbenzamides – prodrugs for amines – stability

Summary

Several 2-hydroxymethylbenzamides derived from various primary and secondary amines were prepared and evaluated as potential prodrug models for the amino group occurring in several drugs. The hydroxy-amides were found to undergo a quantitative cyclization in aqueous solution to phthalide and the parent amine. The lactonization was specific acid- and base-catalyzed as well as subject to buffer catalysis. The structural factor being predominantly responsible for the different reactivities of the hydroxy-amides was found to be the steric properties of the amines, the amine basicity being only of minor importance. The cyclization proceeded only slowly at pH 7.4 and 37°C and in order to be a useful prodrug principle it may be necessary to accelerate the reaction rate, e.g. by introducing sterically or catalytically accelerating substituents in the hydroxy-amide moiety. Acylation of the hydroxymethyl group was shown to block the lactonization and hence to stabilize the hydroxy-amides. However, in the presence of human plasma the ester grouping was readily hydrolyzed yielding the parent 2-hydroxymethylbenzamide. Such cascade latentiation may thus be a particularly useful prodrug principle affording at the same time adequate in vitro stability and in vivo lability.

Introduction

A promising approach to improve drug delivery is chemical transformation of the active drug substances into per se inactive derivatives (prodrugs) which convert to the parent compounds by virtue of enzymic or chemical lability within the body system. A major problem for the general application of this principle is, however, the limited availability of chemical derivative types satisfying the prodrug requirements, the most prominent of these being reconversion of the prodrug to the parent drug in vivo. The three most commonly occurring functional groups in drug molecules are carboxyl, hydroxyl and amino groups. Whereas several ester types have been exploited for utilization in designing prodrugs of carboxylic acids and hydroxy compounds there is a paucity of broadly applicable bioreversible derivatives for amino compounds including peptides (Bundgaard, 1985). Derivative types which have been proposed as prodrugs for amines include N-Mannich bases, enaminones,

* Part 41 of this series: Bundgaard, H., Falch, E., Larsen, C., Mosher, G. and Mikkelson, T.J., Pilocarpic acid esters as novel sequentially labile pilocarpine prodrugs for improved ocular delivery. J. Med. Chem., 28 (1985) 979–981.

Correspondence: H. Bundgaard, The Royal Danish School of Pharmacy, Department of Pharmaceutical Chemistry AD, 2 Universitetsparken, DK-2100 Copenhagen, Denmark.

Schiff bases and, in case of tertiary or N-heterocyclic amines, acyloxyalkyl derivatives (Bundgaard, 1985). N-Acylation of amines to give amide prodrugs has been used only to a limited extent due to the relative stability of amides in vivo although certain activated amides are sufficiently chemically labile and certain amides formed with amino acids may be susceptible to undergo enzymatic cleavage in vivo (for a recent review, see Bundgaard (1985)).

A promising approach to obtain an amide prodrug capable of releasing the parent amine drug at physiological conditions of pH and temperature is to make use of intramolecular chemical catalysis or assistance of the amide hydrolysis. Thus, in several cases neighbouring hydroxyl groups have been reported to facilitate the hydrolysis of amides, e.g. 4-hydroxybutyramide (Bruice and Marquardt, 1962; Martin et al., 1964; Yamana et al., 1972), 4-hydroxybutyranilide (Cunningham and Schmir, 1967), 2-hydroxyalkyl benzenesulfonamides (Wagenaar et al., 1984), some 6-hydroxybicyclo [2.2.1] heptane-2-carboxamides (Morris and Page, 1980a and b), various hydroxy-anilides and phenolic hydroxy-amides (Yamana et al., 1973) and 2-hydroxymethylbenzamides (Belke et al., 1971; Okuyama and Schmir, 1972; Chiong et al., 1975).

The latter derivatives may undergo a relatively rapid cyclization in aqueous solution to give phthalide and free amine (Fig. 1). For prodrug design the rate of lactonization, and hence the rate of amine release, is probably too low at pH 7.4 although only amides of ammonia, methylamine and benzylamine have been studied (Belke et al., 1971; Okuyama and Schmir, 1972; Chiong et al., 1975). However, by substituting the two methylene hydrogen atoms of 2-hydroxymethylbenzamide or by the introduction of steric or catalytic substituents positioned ortho to either the hydroxymethyl or the carboxamide group an acceleration of the rate of lactonization may be achievable (Chiong et al., 1975; Fife and Benjamin, 1974). As suggested by Cain (1976) this prodrug principle may become even more attractive by masking the hydroxyl function in the 2-hydroxymethylbenzamides by acylation to give stable 2-acyloxymethylbenzamides (Fig. 1). In this way the lactonization becomes blocked and must be preceded by hydrolysis of the ester grouping, i.e. by the action

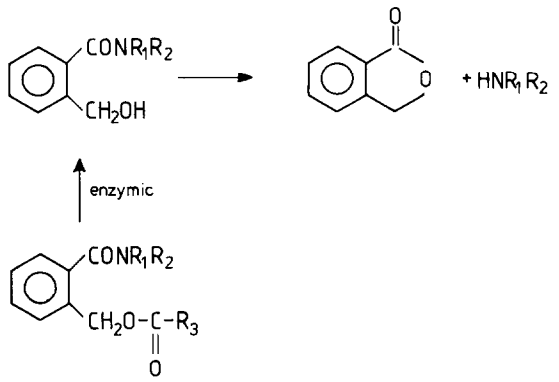


Fig. 1. Principle of the double prodrug concept: conversion of 2-acyloxymethylbenzamides to 2-hydroxymethylbenzamides by enzymic hydrolysis and subsequent cyclization of these to phthalide with release of the parent amine drug.

of esterases in vivo. Besides, by providing in vitro stable derivatives such cascade latentiation would allow the prodrug designer to vary and control the lipophilicity/hydrophilicity of the prodrug by the appropriate selection of the acyl group. While Cain (1976) has reported the synthesis of acetate and benzoate esters of some 2-hydroxymethylbenzamides, information on the stability and enzymatic hydrolysis of such derivatives are not available.

In our laboratory studies have been initiated to explore the utility of this double prodrug concept for drug substances containing an amino group. In the present work a series of 2-hydroxymethylbenzamides of various amines were prepared and their conversion studied in aqueous solution to obtain basal information on the chemical reactivity, including information on the influence of the amine structure on the rate of lactonization. Besides, the chemical- and enzyme-mediated conversion of acylated 2-hydroxymethylbenzamides was investigated.

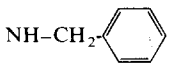
Materials and Methods

Apparatus

^{1}H-NMR spectra were run on a Varian 360L instrument using tetramethylsilane as internal reference. The pH measurements were made at the temperature of study using a Radiometer Type

TABLE 1

CHEMICAL STRUCTURES AND MELTING POINTS OF VARIOUS 2-HYDROXYMETHYLBENZAMIDES AND 2-ACYLOXYMETHYLBENZAMIDES INVESTIGATED IN THIS STUDY

Compound	R_1	R_2	m.p. (°C)
1	$NH-CH_3$	H	123–124 (123–124) [a]
2	$NH-CH_2CH_3$	H	84–85
3	$NH-CH_2CH_2CH_3$	H	74–75
4	$NH-CH(CH_3)_2$	H	110.5–111.5
5	$NH-CH_2CH_2CH_2CH_3$	H	– [c]
6	$NH-CH(CH_3)CH_2CH_3$	H	76–78
7	$NH-CH_2CH(CH_3)_2$	H	79–80
8		H	131–132 (134–135) [b]
9	$N-(CH_3)_2$	H	– [c]
10		H	88–89.5
11		H	108.5–110
12	$NH-CH_2CH_2OH$	H	oil
13	$NH-CH_2CONH_2$	H	83–85
14	$NH-CH_3$	CH_3CO	78–79
15		CH_3CO	71.5–72.5

[a] Chiong et al. (1975).
[b] Belke et al. (1971).
[c] Not isolated in pure form (HPLC analysis indicated the presence of about 15% of phthalide).

PHM 26 instrument. Melting points were taken on a capillary melting-point apparatus and are uncorrected. High-performance liquid chromatography (HPLC) was done with a Spectra-Physics Model 3500B instrument equipped with a variable wavelength detector and a 10-μl loop injection valve. A column, 250 × 4 mm, packed with LiChrosorb RP-8 (7 μm particles) (E. Merck, F.R.G) was used. Microanalyses were performed at Microanalytical Laboratory, University of Copenhagen.

Chemicals

Phthalide and various amines were purchased from A.G. Fluka, Switzerland or E. Merck, F.R.G. Buffer substances and all other chemicals or solvents used were of reagent grade.

Preparation of 2-hydroxymethylbenzamides

The various 2-hydroxymethylbenzamides (1–13; Table 1) were generally prepared by reacting phthalide at room temperature with an excess of the appropriate amine in methanol or ethanol or by using the amine as solvent as described previously for the compounds 1 and 8 (Theilacker and Kalenda, 1953; Belke et al., 1971; Chiong et al., 1975). The progress of the reactions was monitored by TLC (silica gel, toluene-ethyl acetate mixtures) and the reaction times varied from 3 to 72 h. In the following the synthesis of N-propyl-2-hydroxymethylbenzamide (3) is described to illustrate the general procedure. A mixture of phthalide (5.0 g; 37 mmol) and *n*-propylamine (6.0 g; 103 mmol) in 25 ml of methanol was stirred at room temperature for 20 h and then evaporated in vacuo. The residue crystallized upon standing at 4°C overnight and it was finally recrystallized from ethyl acetate.

The 2-hydroxymethylbenzamide derived from glycinamide (13) was prepared in the following manner. A mixture of phthalide (2 g, 15 mmol), glycinamide hydrochloride (3.3 g, 30 mmol) and triethylamine (6.2 ml, 45 mmol) in 20 ml of methanol was stirred at 50°C for 2 h and then evaporated in vacuo. The residue was recrystallized twice from water.

The melting points of the compounds are listed in Table 1. The ^{1}H-NMR spectra of the compounds were consistent with their structure.

Preparation of 2-acyloxymethylbenzamides

N-Methyl-2-acetoxymethylbenzamide (14) was prepared by treating N-methyl-2-hydroxymethylbenzamide (1) (2.5 mmol) with 1 ml of acetic anhydride in 5 ml of pyridine. After stirring the mixture for 1 h at room temperature 30 ml of water was added. The mixture was then extracted with ethyl acetate (2 × 30 ml). The extracts were washed with 2 M hydrochloric acid and water, dried over anhydrous sodium sulphate and evaporated under reduced pressure. The solid residue (14) obtained was recrystallized from ethyl

acetate–petroleum ether, m.p. 78–79°C

Anal. Calcd. for $C_{11}H_{13}NO_3$: C, 63.76; H, 6.32; N, 6.76. Found: C, 63.40; H, 6.30; N, 6.57.

N-Morpholino-2-acetoxymethylbenzamide (15) was prepared in a similar manner from compound 11 and recrystallized from ethyl acetate–petroleum ether, m.p. 71.5–72.5°C

Anal. Calcd. for $C_{14}H_{17}NO_4$: C, 63.87; H, 6.51; N, 5.32. Found: C, 63.58; H, 6.62; N, 5.35.

Kinetic measurements

The degradation of N-methyl-2-hydroxymethylbenzamide (1) was studied in aqueous buffer solutions at 60.0 ± 0.2°C. Hydrochloric acid, acetate, phosphate, borate and carbonate solutions were used as buffers. A constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride. Most other compounds were only studied in aqueous borate buffer solutions (0.02–0.1 M) of pH 9.30 at 60°C.

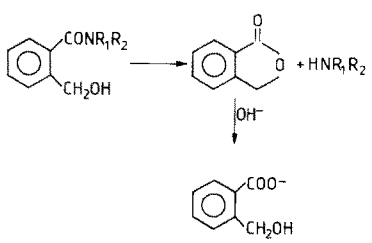
The rates of degradation were followed by using a reversed-phase HPLC procedure. Mobile phase systems of 30–60% v/v methanol in 0.01 M acetate buffer of pH 5.0 were used. The flow rates were 0.8–2.0 ml · min^{-1} and the column effluent was monitored at 240 nm. The elution times for the various compounds were between 4 and 10 min. The compounds were quantified 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 to 10 ml of buffer solution, pre-heated at 60°C, in screw-capped test tubes, the final concentrations in the reaction mixture being about 4×10^{-4} M. The solutions were kept in a water-bath at 60°C and at appropriate times 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 amide derivative against time.

The degradation of the derivatives 11, 14 and 15 was also studied at 37°C in 0.01 M phosphate buffer of pH 7.40 containing 80% human plasma. Initial concentrations of the compounds were about 4×10^{-4} M. At appropriate times samples of 200 μl were withdrawn and added to 1000 μl of ethanol in order to deproteinize the samples. After mixing and centrifugation for 2 min, 10 μl of the clear supernatant was analyzed by HPLC as described above.

Results and Discussion

Lactonization of 2-hydroxymethylbenzamides

The products of degradation of the 2-hydroxymethylbenzamides in acidic solution are the phthalide and the corresponding amine. Analysis of reaction solutions by HPLC revealed the quantitative formation of phthalide as seen from Fig. 2. In neutral and basic aqueous solution the phthalide was found to be formed as an intermediate, the final product being 2-hydroxymethylbenzoate. Kinetic analysis of the time-courses (Fig. 3) for the products of degradation of the hydroxyamide of morpholine (compound 11) showed that in alkaline solution the hydroxy-amides are initially converted into phthalide followed by hydrolysis to the anion of 2-hydroxymethylbenzoic acid in agreement with previous findings (Belke et al., 1971) (Scheme 1). Phthalide is subject to specific base-catalyzed hydrolysis and at 60°C a value of 150 $M^{-1} \cdot min^{-1}$ was determined for the hydroxide ion catalytic rate constant. Phthalide is considerably less stable than most of the hydroxy-amides



Scheme 1.

in alkaline solution and therefore, it does not accumulate during the degradation of these compounds.

Kinetics of lactonization

The kinetics of the degradation of N-methyl-2-hydroxymethylbenzamide (1) was studied at 60°C in the pH-range 1–9.7. At constant pH and temperature strict first-order kinetics was observed for more than 3–4 half-lives. Some typical first-order plots are shown in Fig. 4.

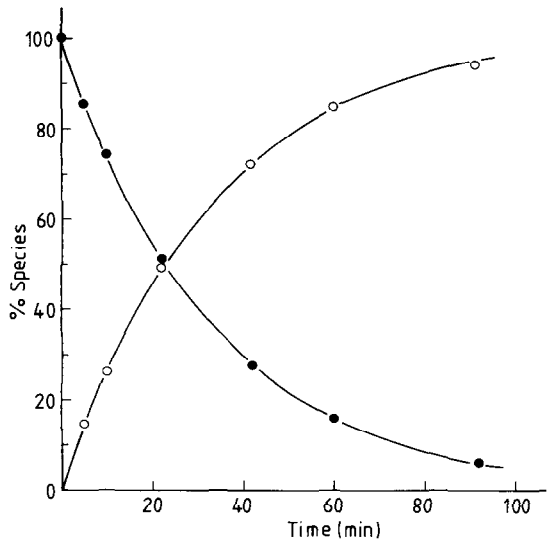


Fig. 2. Time courses for the disappearance of compound 1 (●) and formation of phthalide (○) in 0.1 M hydrochloric acid at 60°C.

The rates of cyclization were subject to pronounced catalysis by the buffer substances used to maintain constant pH. The plots in Fig. 5 give an impression of the magnitude of catalysis. Similar buffer catalysis has been reported before for 2-hydroxymethylbenzamide and N-benzyl-2-hydroxymethylbenzamide (Belke et al., 1971). The buffer-independent pseudo-first-order rate constants (k) were determined by extrapolation of the observed pseudo-first-order rate constants (k_{obs}) vs. total buffer concentration to zero concentration.

The influence of pH on the lactonization of the hydroxy-amide (1) is shown in Fig. 6 where the logarithm of k is plotted against pH. The shape of the pH–rate profile indicates the occurrence of specific acid and base catalysis as well as a spontaneous or water-catalyzed reaction (except in the pH-range 6–8) according to the following rate expression:

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

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 60°C according to the following equation (Harned and Hamer, 1933):

$$\log a_{OH} = pH - 13.02 \quad (2)$$

Values of the second-order rate constants for the specific acid (k_H) and specific base (k_{OH}) cata-

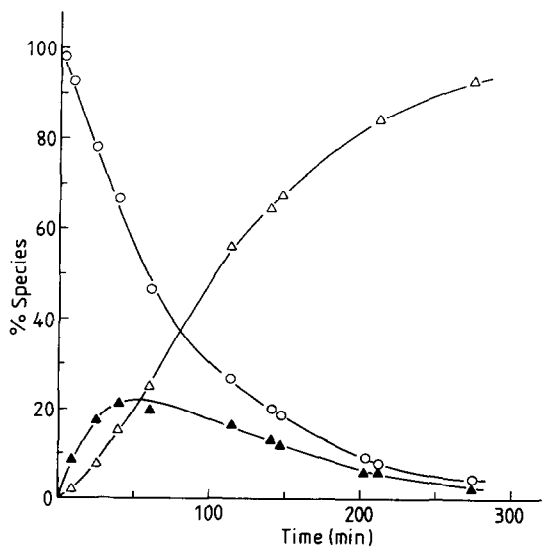


Fig. 3. Time courses for compound 1 (○), phthalide (▲) and 2-hydroxymethylbenzoate (△) during degradation of compound 1 in 0.05 M borate buffer solution of pH 9.30 at 60°C.

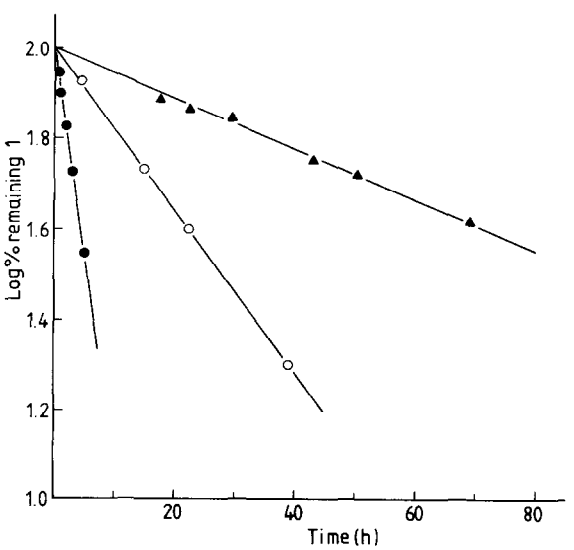


Fig. 4. First-order plots for the lactonization of compound 1 in aqueous buffer solutions of pH 2.0 (●), pH 3.0 (○) and pH 6.0 (▲) at 60°C.

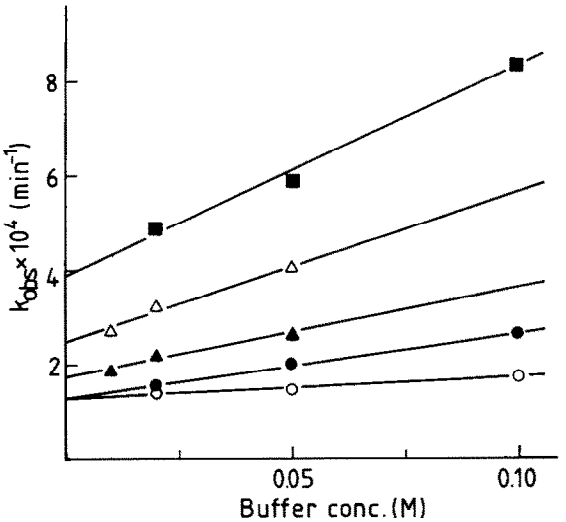


Fig. 5. Effect of buffer concentration on the rate of lactonization of compound 1 at 60°C. Key: ○, acetate pH 5.0; ●, acetate pH 4.0; ▲, phosphate pH 6.9; △, phosphate pH 7.45; ■, borate pH 8.45.

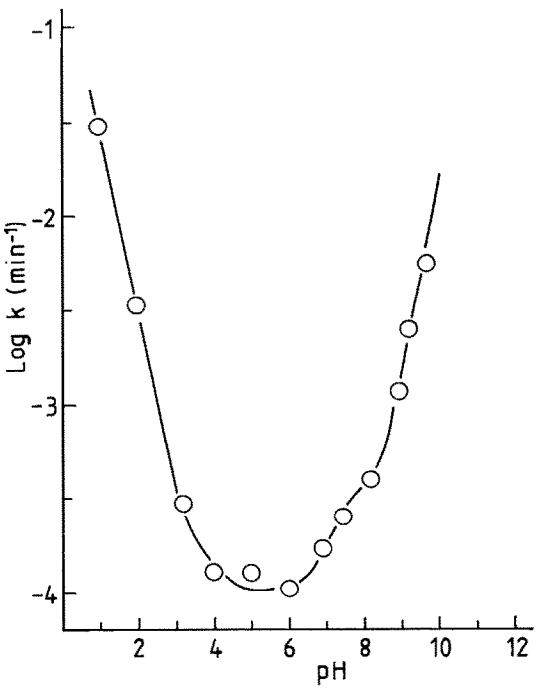


Fig. 6. The pH– rate profile for the degradation of compound 1 in aqueous solution at 60°C.

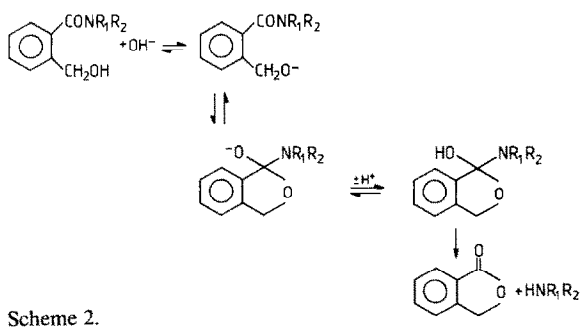
lyzed lactonization were determined from the straight line portions of the pH–rate profile at low and high pH values, respectively, whereas the value of the first-order rate constant for spontaneous cyclization (k_o) was obtained from the rate data at pH 4–6. The following values were derived:

$$k_H = 0.32\ M^{-1} \cdot min^{-1}$$

$$k_{OH} = 12.6\ M^{-1} \cdot min^{-1}$$

$$k_o = 1.1 \times 10^{-4}\ min^{-1}$$

Eqn. 1 does not describe the dependence of k on pH in the pH-range 6–8. As seen in Fig. 6 there is a break in the pH–rate profile at these pH values. As reported previously (Belke et al., 1971; Chiong et al., 1975) this break undoubtedly reflects a change in the rate-determining step. Okuyama and Schmir (1972) have convincingly shown that at pH below neutrality the rate-limiting step is the formation of a tetrahedral intermediate, while at higher pH, intermediate breakdown, i.e. amine expulsion, becomes rate-determining (Scheme 2). The reaction mechanism for the hydroxide ion-catalyzed lactonization most likely involves ionization of the hydroxy group followed by intramolecular nucleophilic attack of the alkoxide ion on the amide.



Scheme 2.

Since the lactonization of the hydroxy-amides is subject to pronounced catalysis by various general acids and bases (Belke et al., 1971; Okuyama and Schmir, 1972) it may be envisaged that the reaction rate would be somewhat higher in vivo, e.g. in the blood. This possibility was tested by determining the rate of lactonization of the derivatives 1 and 11 in 80% human plasma of pH 7.40 at 37°C and in solutions without plasma. The pseudo-

TABLE 2

PSEUDO-FIRST-ORDER RATE CONSTANTS (k_{obs}) AND HALF-LIVES ($t_{1/2}$) FOR THE LACTONIZATION OF THE 2-HYDROXYMETHYLBENZAMIDES 1 AND 11 IN 0.01 M PHOSPHATE BUFFER SOLUTION WITH OR WITHOUT 80% HUMAN PLASMA (AT 37°C)

Compound	Buffer		80% Plasma	
	k_{obs} (h^{-1})	$t_{1/2}$ (h)	k_{obs} (h^{-1})	$t_{1/2}$ (h)
1	1.47×10^{-3}	472	1.42×10^{-3}	488
11	1.41×10^{-2}	49	3.21×10^{-2}	22

first-order rate constants obtained are listed in Table 2. On the basis of these results plasma does not seem to catalyze nor inhibit the cyclization of the hydroxy-amides to any significant extent, at least as determined in vitro.

Structural effects on the rate of lactonization

A main objective of the present study was to examine the influence of the amine structure on the rate of lactonization. To this end the degradation of a number of 2-hydroxymethylbenzamides derived from various amines was compared at pH 9.30 and 60°C. The pseudo-first-order rate constants obtained, after correction for catalysis by the borate buffers used, are listed in Table 3.

The structural factor being predominantly responsible for the different reactivities of the hydroxy-amides appears to be the steric properties of the amines. In Fig. 7 log k (at pH 9.30) has been plotted against Charton's steric parameter, ν (Charton, 1977), for alkyl- and dialkylamino groups. It can be seen that the difference in reactivity of the compounds, excluding the dimethylamide 9, can be correlated reasonably well with the difference in steric effects of the alkyl groups on the amide nitrogen. The regression equation between log k and ν for the eight amides derived from primary amines is given by Eqn. 3:

$$\log k = -2.28\,\nu - 1.74 \qquad n = 8;\ r = 0.916 \qquad (3)$$

Although the reactivity of these 2-hydroxymethylbenzamides decreases uniformly with increasing steric effects within the alkylamino group the hydroxy-amides derived from the secondary amines,

TABLE 3

PSEUDO-FIRST-ORDER RATE CONSTANTS (k) AND HALF-LIVES ($t_{1/2}$) FOR THE LACTONIZATION OF VARIOUS 2-HYDROXYMETHYLBENZAMIDES AT pH 9.30 AND 60°C

Compound	$k\times10^3$ (min^{-1})	$t_{1/2}$ (min)
1	2.6	266
2	1.9	365
3	0.72	513
4	0.16	4331
5	0.49	1415
6	0.063	11,000
7	0.18	3850
8	0.30	2310
9	12.4	56
10	6.0	116
11	9.7	71
12	0.55	1260
13	0.28	2475

dimethylamine (see Fig. 7), morpholine and piperidine show an enhanced reactivity and they are in fact the most reactive of the compounds studied, cf. Table 3. The reason for this steric acceleration is not obvious.

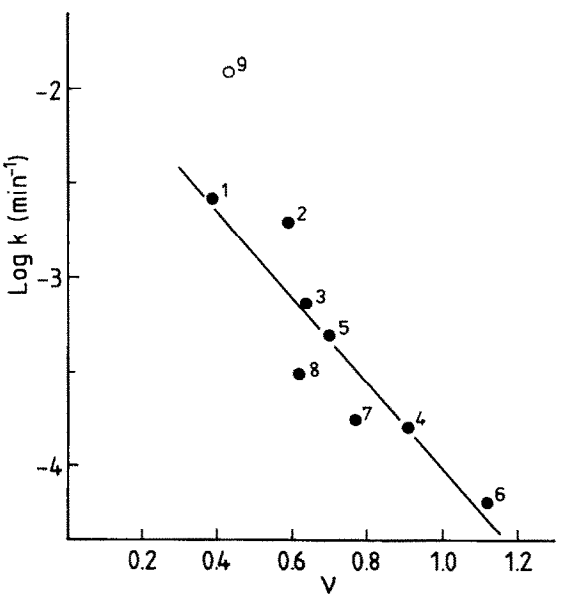


Fig. 7. Plot of log k against the steric substituent parameter ν for the lactonization of compounds 1–9 at pH 9.30 and 60°C. Compound 9 was excluded from the regression plot.

TABLE 4

PSEUDO-FIRST-ORDER RATE CONSTANTS (SCHEME 3) FOR THE OVERALL HYDROLYSIS OF 2-ACETOXYMETHYLBENZAMIDES AT pH 9.30 AND 60°C

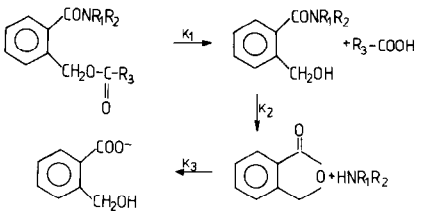
Compound	k_1 (min^{-1})	k_2 (min^{-1})	k_3 (min^{-1})
14	2.1×10^{-2}	2.6×10^{-3}	2.9×10^{-2}
15	2.3×10^{-2}	9.7×10^{-3}	2.9×10^{-2}

The basicity of the parent amine appears to be of little importance. Thus, the reactivity of the glycinamide derivative 13 is almost similar to that of the propylamide derivative 3. The steric properties of the amine entities in these derivatives are not very different but the pK_a of propylamine is 10.7 as compared to 8.1 for glycinamide. Also, the morpholino and piperidino derivatives 10 and 11 show almost the same reactivity despite a great difference in amine basicity (pK_a is 11.1 for piperidine and 8.3 for morpholine).

Hydrolysis of 2-acyloxymethylbenzamides

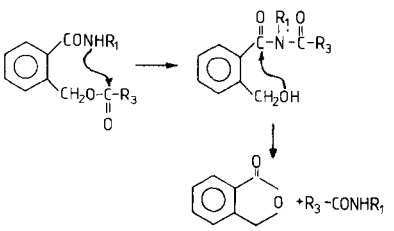
The hydrolytic behaviour of the acetylated derivatives (14 and 15) of N-methyl-2-hydroxymethylbenzamide and N-morpholino-2-hydroxymethylbenzamide was assessed in borate buffer solutions of pH 9.30 at 60°C as well as in plasma solutions at 37°C. Using an HPLC procedure capable of determining both the acyloxy-amide, hydroxy-amide, phthalide and 2-hydroxymethylbenzoate it was possible to obtain time-courses such as those shown in Fig. 8 for compound 15. The acyloxy-amide hydrolyzed according to first-order kinetics to the parent 2-hydroxy-amide which then underwent cyclization to give phthalide and amine, the final product being 2-hydroxymethylbenzoate arising from hydrolysis of phthalide. At any time of reaction the sum of the remaining acyloxy-amide and these products corresponded to 100 ± 3% on a molar concentration basis. This reaction sequence is depicted in Scheme 3 and the pseudo-first-order rate constants derived are given in Table 4 along with those also determined for compound 14. This derivative behaved exactly as compound 15.

These results demonstrate that the lactonization and hence the release of parent amine in fact



Scheme 3.

becomes blocked by acylation of the hydroxymethyl group as suggested in the Introduction. It could be envisaged that the acyloxy-amides derived from primary amines at least to some extent might undergo an intramolecular O → N acyl migration as shown in Scheme 4. Such a reaction would not



Scheme 4.

lead to release of free amine but instead yield an acylated amine. That such a complicating reaction does not occur was proved by several facts, the most obvious one being the formation of the inter-

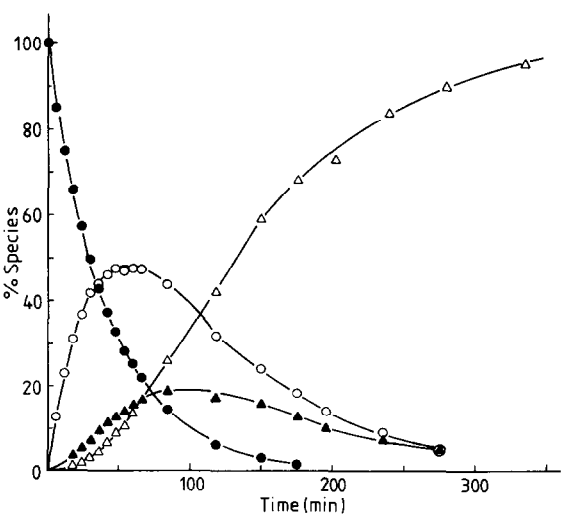


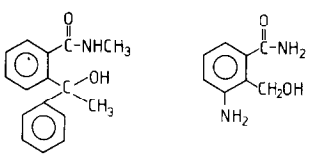
Fig. 8. Time courses for compound 15 (●), compound 11 (○), phthalide (▲) and 2-hydroxymethylbenzoate (△) during degradation of compound 15 in 0.05 M borate buffer solution of pH 9.30 at 60°C

mediate 2-hydroxymethylbenzamide in quantitative amounts.

The ester function in the acyloxy-amides 14 and 15 was as expected found to be subject to enzymatic hydrolysis. Runs performed in 80% human plasma solutions of pH 7.4 and at 37°C revealed a fairly rapid conversion of compounds 14 and 15 to the corresponding hydroxy-amides, the half-lives being 3.2 and 1.4 h, respectively. At similar conditions but in the absence of plasma compounds 14 and 15 hydrolyzed with half-lives of 430 and 335 h, respectively. The conversion of the acyloxy-amides to the corresponding hydroxy-amides proceeded quantitatively in the plasma solutions and the reaction sequence in Scheme 3 is thus also valid under conditions simulating those prevailing in vivo.

Considerations of hydroxy-amides as prodrug forms for amines

The observed rates of lactonization of the 2-hydroxymethylbenzamides appear to be far too low for considering the amides as potentially useful prodrug forms for drug substances containing an amino group. At pH 7.4 and 37°C the half-lives of lactonization of the various derivatives can be estimated to be from 40 h (compound 9) to about 8×10^3 h (compound 6). According to the mechanism of cyclization and the runs performed in plasma the rates might not be expected to change in vivo. The present study shows, however, that in principle, acylation of amines by a hydroxy-acid may be a method of obtaining prodrug forms, especially when combined with acylation of the hydroxy group to provide stable yet enzymatic labile double prodrug forms. The 2-hydroxymethylbenzamides are converted quantitatively to the parent amines and as noted in the Introduction appropriate introduction of substituents in the hydroxymethylphenyl moiety may result in accelerated rates of cyclization. Thus, Chiong et al. (1975) have shown that the hydroxy-amide (17) is about 200-fold more susceptible to undergo both hydrogen ion-, hydroxide ion- and general base-catalyzed lactonization than the corresponding 2-hydroxymethyl derivative (compound 1). As is the case for the acid-catalyzed lactonization of 2-hydroxymethylbenzoic acids (Bunnett and Hauser, 1965) further steric acceleration may certainly be achieved by the introduction of substituents in the ortho position to either the hydroxymethyl or carboxamide group. In fact, the pH-independent cyclization of 3-amino-2-hydroxymethylbenzamide (18) has been reported to proceed at a rate 10^3 times greater than that of unsubstituted 2-hydroxymethylbenzamide (Fife and Benjamin, 1974). At pH 4–10 and 30°C the lactonization of compound 18 showed a half-life of 5–10 min, the large rate enhancement being ascribed to intramolecular general base catalysis by the 3-amino group. Other



17 18

possibilities of obtaining steric and catalytic acceleration of the lactonization are presently being studied in our laboratory and the results will be communicated in a later paper.

Acknowledgement

This work was supported by the Danish Medical Research Council (J. nr. 12-5136).

References

Belke, C.J., Su, S.C.K. and Shafer, J.A., Imidazole-catalyzed displacement of an amine from an amide by a neighbouring hydroxyl group. A model for the acylation of chymotrypsin. J. Am. Chem. Soc., 93 (1971) 4552–4560

Bruice, T.C. and Marquardt, F.H., Hydroxyl group catalysis. IV. The mechanism of intramolecular participation of the aliphatic hydroxyl group in amide hydrolysis. J. Am. Chem. Soc., 84 (1962) 365–370.

Bundgaard, H., Design of prodrugs: bioreversible derivatives for various functional groups and chemical entitites. In H. Bundgaard (Ed.), Design of Prodrugs, Elsevier Science Publishers, Amsterdam, 1985, pp. 1–92.

Bunnett, J.F. and Hauser, C.F., Steric acceleration of the lactonization of 2-(hydroxymethyl)benzoic acids. J. Am. Chem. Soc., 87 (1965) 2214–2220.

Cain, B.F., 2-Acyloxymethylbenzoic acids. Novel amine protective functions providing amides with the lability of esters. J. Org. Chem., 41 (1976) 2029–2031.

Charton, M., The prediction of chemical lability through substituent effects. In Roche, E.B (Ed.), Design of Biopharmaceutical Properties through Prodrugs and Analogs, American Pharmaceutical Association, Washington, DC, 1977, pp. 228–280.

Chiong, K.N.G., Lewis, S.D. and Shafer, J.A., Rationalization of the rate of the acylation step in chymotrypsin-catalyzed hydrolysis of amides. J. Am. Chem. Soc., 97 (1975) 418–423.

Cunningham, B.A. and Schmir, G.L., Hydroxyl group participation in amide hydrolysis. The influence of catalysis on the partitioning of a tetrahedral intermediate. J. Am. Chem. Soc., 89 (1967) 917–922.

Fife, T.H. and Benjamin, B.M., Intramolecular general base catalysed alcoholysis of amides. J. Chem. Soc., Chem. Comm. (1974) 525–526.

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.

Martin, R.B., Hedrick, R. and Parcell, A., Acid hydrolysis of γ-hydroxybutyramide. J. Org. Chem., 29 (1964) 158–160.

Morris, J.J. and Page, M.I., Hydroxy-group participation in the hydrolysis of amides and its effective concentration in the absence of strain effects. J. Chem. Soc. Perkin Trans. II (1980a) 679–684.

Morris, J.J. and Page, M.I., Structure-reactivity relationships and the mechanism of general base catalysis in the hydrolysis of a hydroxy-amide. Concerted breakdown of a tetrahedral intermediate. J. Chem. Soc. Perkin Trans. II (1980b) 685–692.

Okuyama, T. and Schmir, G.L., Hydrolysis of 1-benzylimino-1,3-dihydroisobenzofuran. Implications for the mechanism of lactonization of 2-hydromethylbenzamides. J. Am. Chem. Soc., 94 (1972) 8805–8811.

Theilacker, W. and Kalenda, H., Über isoindole. Justus Liebigs Ann. Chem., 584 (1953) 87–95.

Wagenaar, A., Kirby, A.J. and Engberts, J.B.F.N., Intramolecular nucleophilic catalysis by the neighbouring hydroxyl group in acid-catalyzed benzenesulfonamide hydrolysis. J. Org. Chem., 49 (1984) 3445–3448.

Yamana, T., Tsuji, A. and Mizukami, Y., Studies on the stability of amides. IV. Intramolecular hydroxyl group participation in the acidic hydrolysis of aliphatic amides. Chem. Pharm. Bull., 20 (1972) 1217–1229.

Yamana, T., Tsuji, A. and Mizukami, Y., Stabilization of drugs. I. The quantitative prediction of the pH-dependency of amide and anilide hydrolyses by neighbouring hydroxyl groups. Chem. Pharm. Bull., 21 (1973) 721–728.