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Prodrugs as drug delivery systems. 77. Phthalidyl derivatives as prodrug forms for amides, sulfonamides, carbamates and other NH-acidic compounds

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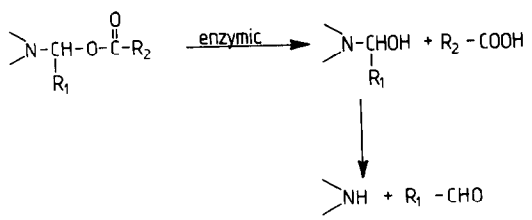
Summary

A series of *N*-phthalidyl derivatives of various carboxamides, sulfonamides, a carbamate and a urea has been prepared with the aim of assessing their potential as prodrug forms for NH-acidic compounds. The hydrolysis of the compounds was studied in aqueous solution at various pH values and in the presence of human plasma. The degradation was shown to take place by hydrolytic opening of the lactone ring with the formation of an *N*-hydroxyalkyl intermediate which quickly decomposed to the parent NH-acidic compound and phthalaldehydic acid. This hydrolysis was catalyzed by plasma enzymes. The phthalidyl derivative of a primary sulfonamide behaved differently as it was extremely unstable in aqueous solution, the decomposition proceeding via an elimination–addition mechanism. It is concluded that in contrast to unstable linear *N*-acyloxyalkyl derivatives, phthalidyl derivatives in which the ester function is incorporated in a lactone group may be useful as prodrug forms for primary as well as secondary amides and other similar NH-acidic compounds, the exception being primary sulfonamides.

Introduction

In recent years *N*-acyloxyalkylation has become a commonly used approach to obtain bioreversible derivatives (prodrugs) of various amides, imides, hydantoins, uracils, tertiary or *N*-heterocyclic amines and other NH-acidic compounds (Bodor, 1981; Pitman, 1981; Bundgaard, 1985a and b). By varying the acyl portion of such *N*-acyloxyalkyl 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. The derivatives in general combine a high in vitro stability with enzymatic lability. The regeneration of the parent drug occurs via a two-step reaction, enzymatic cleavage of the ester moiety followed by spontaneous decomposition of the *N*-hydroxyalkyl derivative (Scheme 1).



Scheme 1.

Although the stability behavior of *N*-acyloxyalkyl derivatives has usually been regarded to be similar to that of other carboxylic acid

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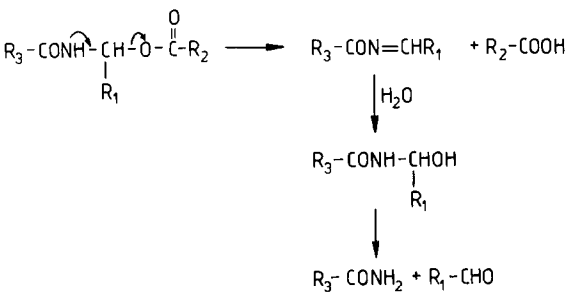
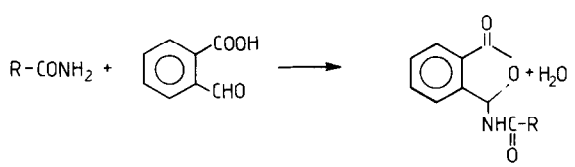
esters, we have recently found that this is not always the case and that the crucial structural factor determining both the rate and mechanism of hydrolysis of *N*-acyloxyalkyl derivatives formed from amides is the nature of the amide, i.e. whether it is a primary or secondary amide (Bundgaard and Nielsen, 1987). Whereas *N*-acyloxyalkyl derivatives of imides and secondary amides as well as of ring structures containing such moieties (e.g. uracils and hydantoins) showed the normal ester stability, *N*-acyloxyalkyl derivatives of primary amides and, by analogy, other primary amide-type structures, were found to be extremely unstable in aqueous solution and to decompose by an elimination–addition mechanism involving a reactive *N*-acylimine intermediate (Scheme 2) (Nielsen and Bundgaard, 1987; Bundgaard and Nielsen, 1987).



Scheme 2.

In this paper, we show, however, that by incorporating the ester group in a lactone function it is possible to obtain *N*-acyloxyalkyl derivatives of primary amides and analogous structures with the desired combination of adequate chemical stability and enzymatic lability.

The derivatives studied (Table 1) are 3-phthalidyl derivatives obtained by condensing amides and other NH-acidic compounds such as a urea, a carbamate and sulfonamides with phthalaldehydic acid (2-formylbenzoic acid) as shown in Scheme 3 for the reaction with a primary amide.



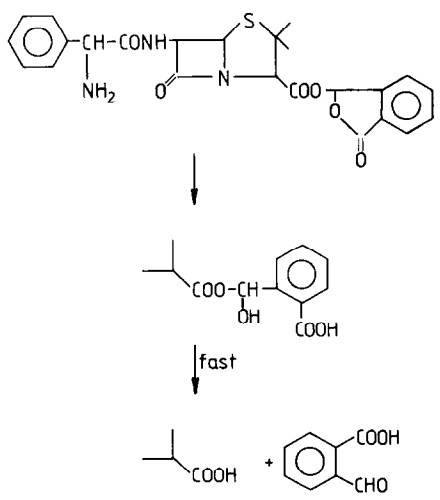
Scheme 3.

TABLE 1

Chemical structures and melting points of various 3-substituted phthalides investigated in this study

Compound no.	R	m.p. (°C)
1	$-NHCOCH_3$	217–218 [a]
2	$-NHCOCH_2Cl$	169–171
3	$-NHCOCHCl_2$	203–204
4	$-NHCOC_6H_5$	175–177 [a]
5	$-NHCO-4-ClC_6H_5$	216–218
6	$-NHCO-4-NO_2C_6H_5$	> 270
7	$-NHCO-4-CH_3OC_6H_5$	200–201
8	$-NHCO-2-OHC_6H_5$	176–177
9		145–146
10	$-NHCOOC_2H_5$	170–171 [a]
11	$-NHCONHCH_3$	227–228 [a]
12	$-NHSO_2C_6H_5$	178–180 [a]
13	$-NSO_2C_6H_5-4-CH_3$ CH_3	177–179

[a] The melting points of these previously described compounds agreed with those reported by Wheeler et al. (1957).



Scheme 4.

The phthalidyl functionality has already been used as a promoiety in the ampicillin prodrug talampicillin (Clayton et al., 1974; 1976; Shiobara et al., 1974; Isaka et al., 1976). The lactone ring in this derivative is readily cleaved by enzymatic hydrolysis with ampicillin and phthalaldehydic acid being spontaneously released from an unstable *O*-acyloxyalkyl intermediate (Scheme 4) (Clayton et al., 1976; Shiobara et al., 1974; Jeffery et al., 1978). As shown below a similar cleavage mechanism is operating for phthalidyl derivatives of certain NH-acidic compounds.

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 (5 μm particles). Microanalyses were performed at the Microanalytical Laboratory, Leo Pharmaceutical Products, Ballerup, Denmark.

Chemicals

Phthalaldehydic acid and various amides were purchased from A.G. Fluka, Switzerland or E. Merck, F.R.G. *N*-Methyl-*p*-toluenesulfonamide was obtained from Aldrich. Buffer substances and solvents used in the kinetic experiments were of reagent grade.

Preparation of the derivatives

The 3-substituted phthalides were prepared by condensing phthalaldehydic acid (which predominantly exists in the cyclized form as 3-hydroxyphthalide (Kagan, 1967)) with equimolar amounts of the appropriate amide, carbamate, urea or sulfonamide essentially as described by Wheeler et al. (1957). Although these authors noted that the reaction of phthalaldehydic acid with amides apparently is limited to amides in which the amide nitrogen is unsubstituted since *N*-methylacetamide failed to react, we observed a ready condensation of phthalaldehydic acid with 2-pyrrolidone, a secondary amide, as well as with *N*-methyl-*p*-toluenesulfonamide. The appropriate compounds (**9** and **13**) were obtained by heating a mixture of equimolar amounts of phthaldehydic acid and 2-pyrrolidone or *N*-methyl-*p*-toluenesulfonamide to 130 °C for 3 h. After cooling to 90–100 °C the mixture was poured into cold water whereupon compounds **9** and **13** precipitated.

All the compounds were recrystallized from ethanol or ethanol–water except compound **6** which was recrystallized from dimethylsulfoxide. The melting points of the compounds are listed in Table 1. All new 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 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 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 50 μl of stock solutions of the derivatives in acetonitrile or dioxane to give a final concentration of about 10^{-4} M. The rate of hydrolysis of the compounds was followed by monitoring the change in absorbance at a wavelength where the absorbances of the substrates and products differed maximally. The wavelengths used were 255 nm (compounds **1**–**8** and **10**), 285 nm (compound **11**), 295 nm (compound **12**) and 258 nm (compound **13**). Pseudo-first-order rate constants were determined from the slopes of lin-

ear plots of $\log(A_t - A_\infty)$ or $\log(A_\infty - A_t)$ vs time, where A_t and A_∞ are the absorbance readings at time t and infinity, respectively.

In all cases where the half-lives of degradation exceeded a few minutes the rates of degradation were followed by using reversed-phase HPLC procedures. Mobile phase systems of 10–60% v/v methanol in 0.01 M acetate buffer of pH 5.0 or mixtures of methanol, acetonitrile and the acetate buffer were generally used, the composition of the solvent being adjusted for each compound to give an appropriate retention time (2–5 min) and to allow separation of the starting material, phthalaldehydic acid and the parent NH-acidic compound. For the specific determination of phthalaldehydic acid a mobile phase system consisting of methanol-0.02 M phosphate buffer pH 3.0(3 : 2 v/v) was used. The column effluent was monitored at 215 or 230 nm and the flow rate was 0.8–1.2 ml/min. 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 acetonitrile or dioxane to 10 ml of preheated buffer solution in screw-capped test tubes, the final concentration of the compounds being about 5×10^{-5} M. The solutions were kept in a water bath at constant temperature 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.

The degradation of several derivatives 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 $1\text{–}2 \times 10^{-4}$ M. At appropriate times samples of 250 µl were withdrawn and mixed with 250 µl of a 20% aqueous solution of trichloroacetic acid in order to deproteinize the samples. After centrifugation at 12,000 rpm for 2 min, 20 µl of the clear supernatant was analyzed by HPLC as described above. In the case of the sulfonamide derivatives **12** and **13** the 250 µl plasma samples were deproteinized by mixing with 500 µl of a 2% solution of zinc sulphate in methanol–water (1 : 1 v/v).

Results and Discussion

Kinetics of decomposition of compounds 1–13

The kinetics of breakdown of the phthalidyl derivatives **1–13** was studied in aqueous solution over a wide range of pH. At constant pH and temperature the disappearance of the compounds displayed strict first-order kinetics over several half-lives. Some typical first-order plots are shown in Figs. 1 and 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 thus obtained agreed within ±3%.

The rates of decomposition were found to be independent of buffer concentration at the concentration used (0.01–0.03 M).

The influence of pH on the degradation rate is shown in Figs. 3–5 where the logarithms of the observed pseudo-first-order rate constants (k_{obs}) are plotted against pH. The benzamide derivative **4** was studied over a wide pH range at 37 °C whereas the other 3-amidophthalides were only studied at pH 6–11. The shape of the pH–rate profile for compound **4** as shown in Fig. 3 indicates the occurrence of specific acid and base catalysis as well as a spontaneous or water-cata-

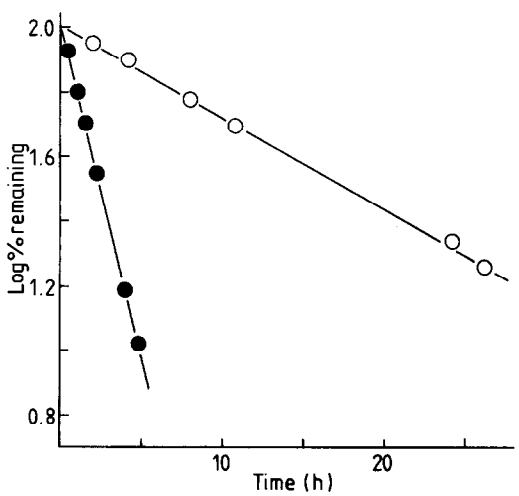


Fig. 1. First-order kinetic plots for the degradation of 3-benzamidophthalide (**4**) in aqueous buffer solutions of pH 7.40 (○) and pH 8.90 (●) at 37 °C.

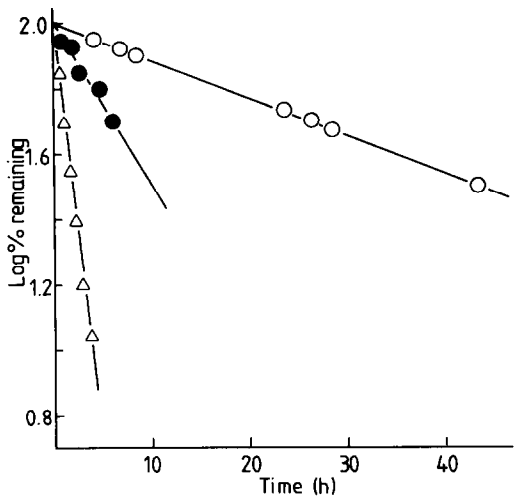


Fig. 2. First-order kinetic plots for the degradation of 3-(*N*-methyl-*p*-toluenesulfonamido)phthalide (**13**) in aqueous buffer solutions of pH 7.40 (○) and pH 9.10 (△) and in 80% human plasma solutions of pH 7.40 (●) at 37 °C.

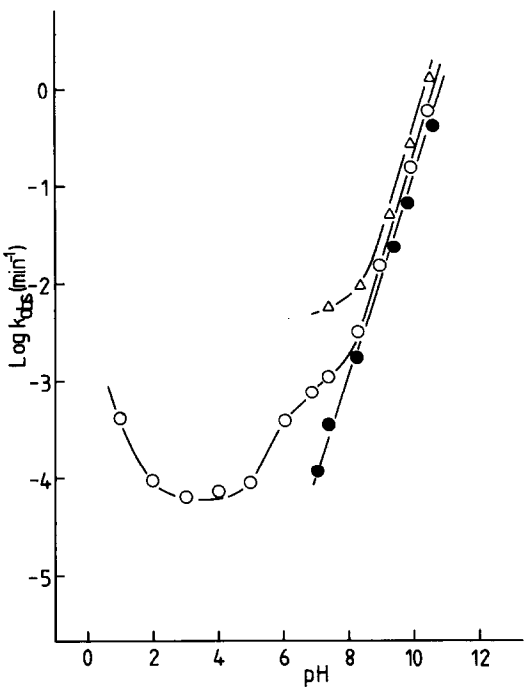


Fig. 3. The pH–rate profiles for the degradation of compound **4** (○), compound **9** (●) and compound **10** (△) in aqueous solution ($\mu = 0.5$) at 37 °C.

TABLE 2

*Second-order rate constants (k_{OH}) for the specific base-catalyzed hydrolysis of the 3-phthalidyl derivatives **1–13** in aqueous solution at 37 °C*

Compound no.	k_{OH} (M^{-1} min^{-1})	σ^* [a]
1	280	0.00
2	1260	0.94
3	5770	1.94
4	630	0.75
5	810	0.87
6	1780	1.26
7	600	0.60
8	1045	
9	450	
10	2340	1.68
11	235	
12	1.2×10^5; 1.6×10^8 [b]	
13	420	

[a] The Taft polar substituent constants σ^* refer to R^1 in **16** and were taken from Perrin et al. (1981).
[b] These values refer to k_{OH} and k^1_{OH}, cf. Table 3.

lyzed reaction (except in the pH range 5.5–8.5) 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):

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

For compound **4** the following values of the rate constants k_H and k_0 were found: $k_H = 4 \times 10^{-3}$ M^{-1} min^{-1} and $k_0 = 5.5 \times 10^{-5}$ min^{-1}. Values of the second-order rate constants for the specific base (k_{OH})-catalyzed degradation were determined from the straight line portions of the pH–rate profiles at high pH values (pH 9.5–11) and are listed in Table 2 for all compounds.

Eqn. 1 does not describe the dependence of k_{obs} on pH in the pH range 5.5–8.5. As seen in Fig. 3 there is a break in the pH–rate profile at these pH values. This could be due to ionization of the substrate but neither spectrophotometric or

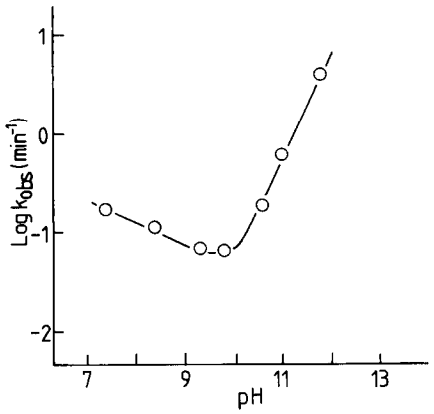


Fig. 4. The pH–rate profile for the degradation of compound **11** in aqueous solution ($\mu = 0.5$) at 37 °C.

titrimetric examinations of compound **4** revealed any indication of an ionization in this pH-range. The break in the pH–rate profile was observed for all the 3-amidophthalides except for compound **9**. The latter is derived from the secondary amide 2-pyrrolidone and consequently, the increased reactivity of the phthalidyl derivatives of the primary amides at neutral pH values may be linked to the NH-moiety.

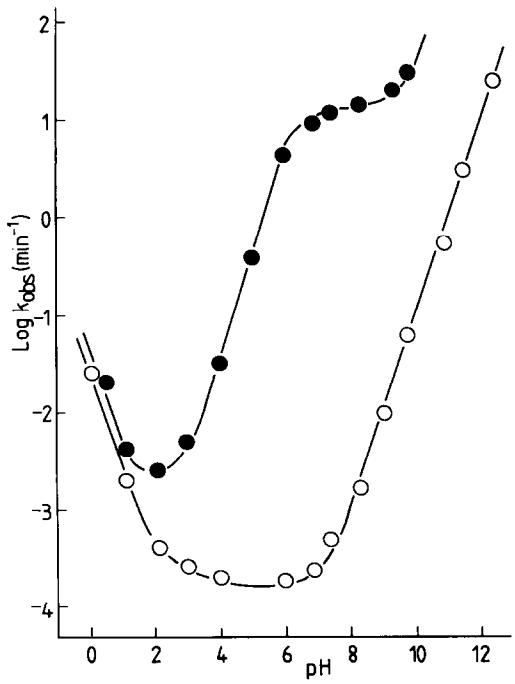


Fig. 5. The pH–rate profiles for the degradation of compound **12** (●) and compound **13** (○) in aqueous solution ($\mu = 0.5$) at 37 °C.

The urea derivative **11** showed a pH-rate profile (Fig. 4) different from the other compounds and not easily accounted for in terms of simple acid and base catalysis. A study is in progress to investigate the hydrolytic characteristics of this compound and other urea derivatives in more detail.

The sulphonamide derivatives **12** and **13** differed greatly in stability except at very low pH values with compound **12** derived from a primary sulfonamide being very reactive. The pH-rate profile for compound **13** (Fig. 5) can be fully accounted for by Eqn. 1 whereas that of compound **12** (Fig. 5) may be described by the following equation:

$$k_{obs} = (k_0 + k_H a_H + k_{OH} a_{OH}) \frac{a_H}{a_H + K_a} + k^1_{OH} a_{OH} \frac{K_a}{a_H + K_a} \quad (3)$$

where $a_H/(a_H + K_a)$ and $K_a/(a_H + K_a)$ are the fraction of the unionized and ionized form of the compound, respectively, k_{OH} and k^1_{OH} are the specific base-catalytic rate constants for these species and K_a is the apparent ionization constant of the compound. The values of the different rate constants and pK_a as obtained by curve-fitting on the basis of Eqns. 1 and 3 are listed in Table 3. The kinetically derived pK_a value for compound 12 of 6.6 appears to be in the expected range. *p*-Toluenesulfonamide has a pK_a value of 10.2 at

TABLE 3

*Rate data for the degradation of the sulfonamidophthalides **12** and **13** in aqueous solution at 37 °C and $\mu = 0.5$*

Compound no.	k_H (M^{-1} min^{-1})	k_0 (min^{-1})	k_{OH} (M^{-1} min^{-1})	k^1_{OH} (M^{-1} min^{-1})
12	0.060	1.5×10^{-3}	1.6×10^{8}	1.2×10^{5}
13	0.031	1.8×10^{-4}	420	

[a] The kinetically determined pK_a value is 6.6.

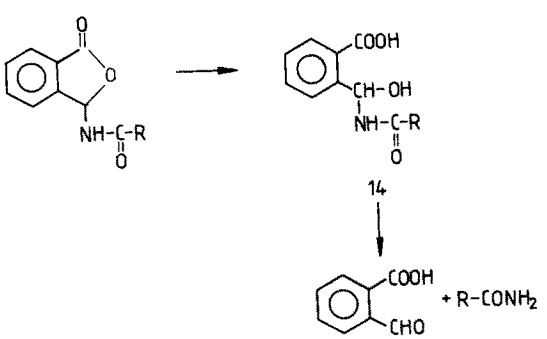
25 °C (Willi, 1956) and the electron-withdrawing phthalidyl substituent should decrease this value considerably. Thus, for compound **12** the break in its pH–rate profile occurring at pH 6–8 may reasonably be ascribed to ionization of the substrate and different reactivities of the ionized and unionized forms.

Products of reaction

The decomposition of the derivatives **1**–**13** proceeded with the quantitative formation of the parent NH-acidic compound and phthalaldehydic acid as revealed by HPLC analysis of the reaction solutions. Examples of a product analysis are shown in Fig. 6 for 3-benzamidophthalide at pH 9.35 and in Fig. 7 for 3-acetamidophthalide in 1 M hydrochloric acid at 60 °C. As can be seen the rate of formation of both benzamide and phthalaldehydic acid followed strict first-order kinetics with no occurrence of any lag period. Similar findings were obtained in product analysis studies of the other compounds investigated.

Mechanism of decomposition

Except for compound **12** the phthalidyl derivatives studied show a chemical reactivity which can be accounted for in terms of a reaction mechanism involving hydrolytic opening of the lactone ring as the rate-determining step in the overall decomposition in aqueous solution. The *N*-hydroxybenzyl derivative (**14**) thus formed (Scheme 5) is expected to decompose much faster than it is formed to produce phthalaldehydic acid and the parent amide-type compound. The observed lack of any lag period in the formation of these final products as described above is in accordance with this reaction scheme. Further support is provided by



Scheme 5.

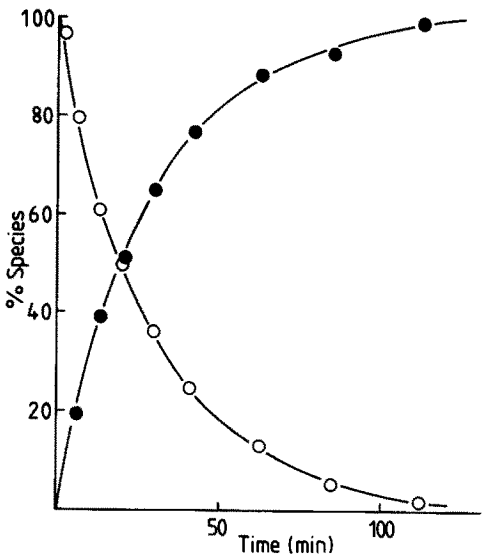


Fig. 6. Time-courses for 3-benzamidophthalide (**4**) (○) and benzamide (●) in the degradation of compound **4** at pH 9.35 at 37 °C.

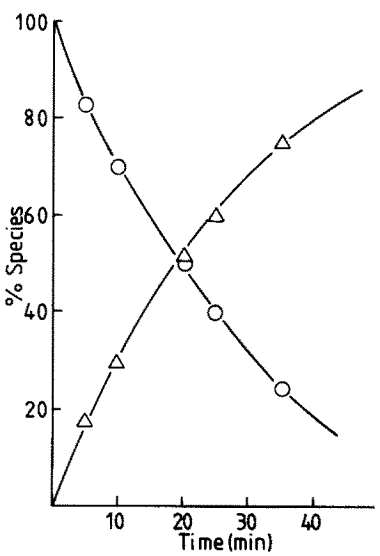
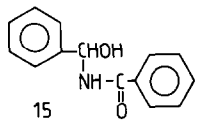


Fig. 7. Time-courses for 3-acetamidophthalide (**1**) (○) and phthalaldehydic acid (△) in the degradation of compound **1** in 1 M hydrochloric acid at 60 °C.

the behaviour of *N*-(α-hydroxybenzyl)benzamide (**15**) which is structurally very similar to the sug-



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gested intermediate derived from 3-benzamidophthalide. Compound **15** has previously been shown to degrade to benzamide and benzaldehyde in aqueous solution at rates which greatly exceed those for the degradation of compound **4** at any pH value (Bundgaard and Johansen, 1984). The k_H and k_{OH} values for compound **15** at 37 °C are 7.9 and 1.8×10^5 M^{-1} min^{-1}, respectively, and at pH 7.4, for example, the half-life of decomposition of compound **15** is 6.5 min whereas that of compound **4** is 640 min. Thus, by assuming that the reactivity of compound **14** derived from benzamide is not very different from that of compound **15** there should be no lag period in the formation of phthalaldehydic acid and the parent NH-acidic compounds as in fact also observed experimentally. By speeding up the lactone ring opening step by virtue of enzymatic catalysis it is, on the other hand, possible to obtain data directly indicating the existence of an intermediate in the overall decomposition in accordance with Scheme 5 (see below).

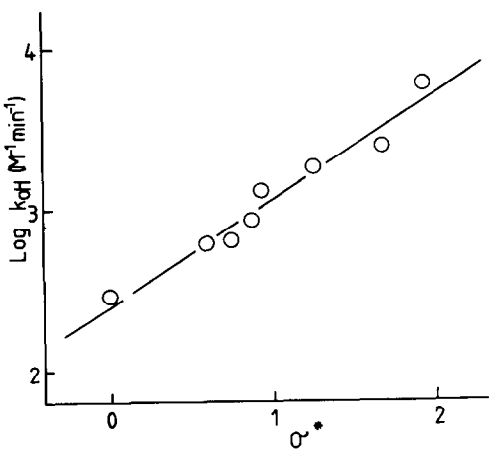
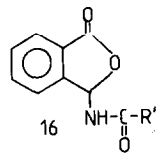


Fig. 8. Plot of log k_{OH} against the Taft polar substituent parameter, σ^*, for compounds **1–7** and **10**. The data are from Table 2.

According to the suggested mechanism of degradation the leaving ability of the alcohol part of the lactones should influence the stability. This is also the case. As seen in Fig. 8 a linear correlation exists between log k_{OH} and the Taft polar substituent parameter σ^*, the latter referring to R' in formula **16**, for the phthalidyl compounds derived



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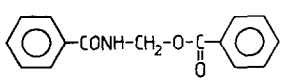
from amides (compound **1–7**) and a carbamate (compound **10**). The regression equation is given by:

$$\log k_{OH} = 0.65(\pm 0.05)\sigma^* + 2.40(\pm 0.06)(n=8;\ r=0.980) \quad (4)$$

As noted in the Introduction *N*-acyloxyalkyl derivatives of primary amides are extremely unstable in aqueous solution. Thus, the k_{OH} value for *N*-(benzoyloxymethyl)benzamide (**17**) at 37 °C is



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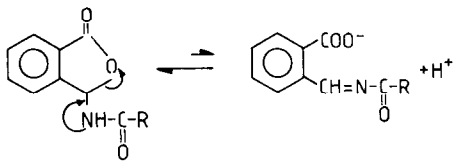
2×10^6 M^{-1} min^{-1} (Bundgaard and Nielsen, 1987). The mechanism responsible for this high reactivity was suggested to involve an unimolecular elimination–addition process with the formation of a transient *N*-acylimine intermediate (Scheme 2) (Bundgaard and Nielsen, 1987). Except for the sulfonamide derivative **12** the phthalidyl derivatives do not show such a high reactivity, thus rendering an elimination-addition mechanism unlikely. This is further substantiated by the reactivity observed for the 3-amidophthalide derived from the secondary amide 2-pyrrolidone (compound **9**). This compound which is structurally incapable of producing an *N*-acylimine shows a k_{OH} value of the same order of magnitude as those for the 3-amidophthalides derived from primary amides. A possible explanation for the different reactivities of the cyclic amidophthalides and linear *N*-acyloxyalkyl amides

may be that the formation of an *N*-acylimine intermediate in the former compounds is unfavourable due to intramolecular attack by the carboxy group on the neighbouring acylimine function as depicted in Scheme 6.

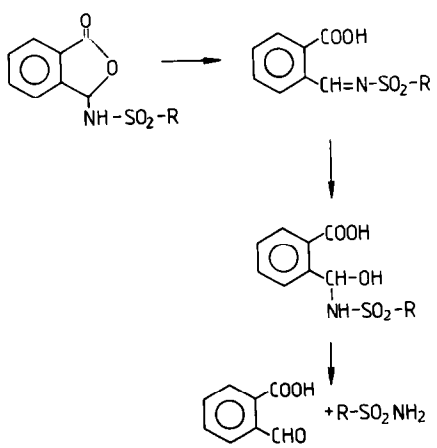


Scheme 6.

For the sulfonamide derivative **12**, on the other hand, an elimination–addition mechanism involving the formation of an *N*-sulfonylimine intermediate is most certainly operating (Scheme 7). Its reactivity is considerably higher than that of the amidophthalides and of the same size as that of linear *N*-acyloxyalkyl amides. Furthermore, the *N*-methyl analog (compound **13**) which is unable to produce an *N*-sulfonylimine intermediate is much more stable in aqueous solution (cf. Fig. 5). The stronger electronegativity of the sulfonyl group relative to that of an acyl group may apparently favour the elimination–addition mechanism.

Hydrolysis in plasma

The rates of decomposition of some of the phthalidyl derivatives were determined in 80% human plasma (pH 7.4) and 37 °C. For all compounds investigated good first-order kinetics for



Scheme 7.

TABLE 4

Half-lives for the degradation of various 3-phthalidyl derivatives in aqueous buffer solution and in 80% human plasma (pH 7.4) at 37 °C

Compound	Half-lives (min)	
	pH 7.4 buffer	80% plasma
1	435	180
4	640	1.7
8	460	4.1
9	1800	900
10	115	14.5
11	5.0	1.6
12	3.5 s	–
13	1585	360

the disappearance of the starting material was observed as exemplified by the plot in Fig. 2 for compound **13**. The rate data obtained are shown in Table 4. It is readily seen that plasma enzymes catalyze the rate of degradation, the extent of catalysis being dependent on the 3-substituent in the phthalides. The 2-pyrrolidone compound **9** is considerably less suceptible to undergo enzymatic catalysis than the amide derivatives 4 and 8 which may be ascribed to greater steric hindrance in compound **9**. Plasma-catalyzed hydrolysis of phthalidyl derivatives of penicillins, e.g. talampicillin (see Scheme 4), has previously been described (Clayton et al., 1976; Isaka et al., 1976), the effect being due to catalysis of hydrolytic cleavage of the lactone ring. The plasma-catalyzed degradation of the amidophthalides may similarly be ascribed to hydrolysis of the lactone moiety (the first step in Scheme 5). Product analysis studies made on the reaction of the 3-benzamidophthalide (**4**) provided strong support for this. In 80% human plasma solutions compound **4** disappeared quickly, the half-life being 1.7 min. However, the formation of the final products of decomposition, benzamide and phthalaldehydic acid, did not follow first-order kinetics but displayed a marked induction period. This is shown in Fig. 9 for benzamide. HPLC analysis of the reaction solution revealed a similar behaviour of phthalaldehydic acid. The lag period observed in the formation of these products indicates the pres-

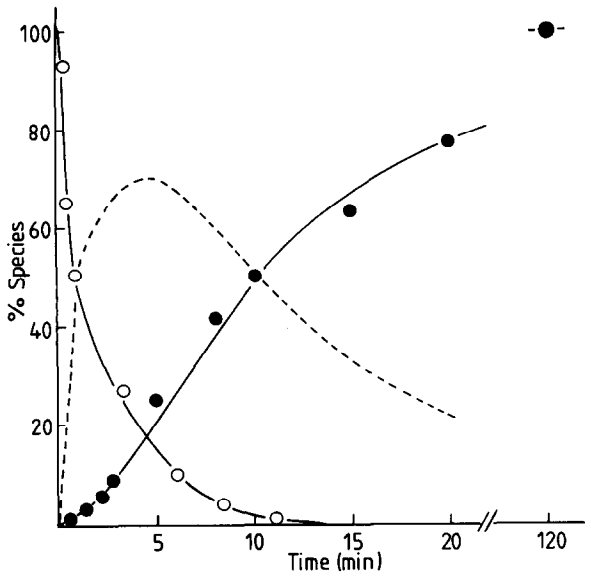


Fig. 9. Time-courses for 3-benzamidophthalide (**4**) (○) and benzamide (●) in the degradation of compound **4** in 80% human plasma (pH 7.4) at 37 °C. The dotted curve was constructed from Eqn. 6.

ence of an intermediate which as discussed above is suggested to be the *N*-hydroxyalkyl derivative **14** (Scheme 5). According to Scheme 5 the expression for the time-course of benzamide arising during the degradation is:

$$\text{mol\% benzamide} = 100 \times \left(1 - \frac{k_2 e^{-k_1 t} - k_1 e^{-k_2 t}}{k_2 - k_1}\right) \quad (5)$$

where k_1 and k_2 are pseudo-first-order rate constants for the first and second steps, respectively, in Scheme 5. In Fig. 9 the curve for benzamide is constructed on the basis of this equation and the following values of the rate constants: $k_1 = 0.42$ min^{-1}; $k_2 = 0.085$ min^{-1}. In Fig. 9 the time course for the intermediate is also shown; it was constructed on the basis of the following equation:

$$\text{mol\% intermediate} = \frac{k_1 \times 100}{k_1 - k_2}(e^{-k_2 t} - e^{-k_1 t}) \quad (6)$$

From the k_2-value a half-life of degradation of the intermediate is calculated to be 8.2 min. This value is seen to be close to the half-life of 6.5 min previously observed for the degradation of the structurally analogous compound **15** at pH 7.4 and 37 °C (Bundgaard and Johansen, 1984). Although the half-life of 8.2 min for the degradation of the postulated intermediate **14** (R = phenyl) is for plasma solution the same value is expected in pure buffer solution since the rate of decomposition of compound **15** or other *N*-hydroxylalkyl amides is not affected significantly by the presence of plasma (Johansen and Bundgaard, 1981; Bundgaard and Johansen, 1984; Bundgaard and Buur, 1987).

Several attempts were made to observe the intermediate by HPLC analysis of the reaction solution of compound **4** but only peaks corresponding to compound **4**, benzamide and phthalaldehydic acid were observed using various solvent systems.

Conclusions

The results obtained suggest that phthalidyl derivatives may be useful as prodrug forms for primary as well as secondary amides and other NH-acidic compounds. In contrast to linear *N*-acyloxyalkyl derivatives of primary amides and similar structures the phthalidyl derivatives in which the ester function is incorporated in a lactone group show a good stability in aqueous solution and at the same time ability to be readily hydrolyzed enzymatically with a quantitative release of the parent amide-type compound. Phthalidyl derivatives of primary sulfonamides are, however, too unstable to be considered as useful prodrug forms but the approach may, on the other hand, be applied to secondary sulfonamides such as *N*-methylsulfonamides which as discussed previously (Larsen and Bundgaard, 1987) may function as a prodrug form for the corresponding primary sulfonamides due to demethylation occurring in vivo.

The phthalidyl group has recently been used as a promoiety in 5-fluorouracil prodrugs (Kametani et al., 1982; Kamata et al., 1985). The *N*-phthalidyl 5-fluorouracil derivatives described were shown to be cleaved in vivo with release of the NH-acidic 5-fluorouracil, the hydrolysis being catalyzed by

liver enzymes in particular (Tonda et al., 1987). The phthalidyl group has also been attached to the imidazole moiety of theophylline and was shown to be readily split off after incubation of the derivative in various rat tissue homogenates (Tonda and Hirata, 1987). In assessing the utility of the phthalidyl group as a carrier in prodrugs of NH-acidic compounds it should finally be pointed out that it is readily possible to control the lipophilicity and water solubility of the derivatives by introducing appropriate substituents in the benzene ring of the phthalidyl group. Thus, the introduction of a carboxylic acid function may be useful if an increased water solubility at physiological pH is desired.

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