International Journal of Pharmaceutics, 71 (1991) 31–43

ADONIS 037851739100176Z

IJP 02379

N-Acyl derivatives as prodrug forms for amides: Chemical stability and enzymatic hydrolysis of various *N*-acyl and *N*-alkoxycarbonyl amide derivatives

Anne H. Kahns and Hans Bundgaard

The Royal Danish School of Pharmacy, Department of Pharmaceutical Chemistry, Copenhagen (Denmark)

(Received 11 December 1990)
(Accepted 28 December 1990)

Key words: Prodrug; *N*-Acylated amide; *N*-Acyl carbamate ester; Hydrolysis; Enzymatic hydrolysis

Summary

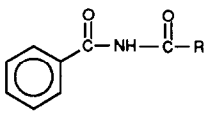
The hydrolysis kinetics and enzymatic cleavage of various *N*-acyl and *N*-alkoxycarbonyl derivatives of benzamide, *N*-methylbenzamide and salicylamide, used as models for the carboxamide group, were studied to assess their suitability as prodrugs for the amide group occurring in various drug substances and bioactive peptides. The pH-rate profiles for the hydrolysis of the derivatives were obtained at 37°C and the products of hydrolysis determined by HPLC analysis. *N*-Acylated amides (diacylamines) were found to be relatively stable in aqueous solution but readily hydrolyzed enzymatically by human plasma. The amount of parent amide formed upon both chemical and enzymic hydrolysis was shown to depend on the nature of the *N*-acyl group. The *N*-acyloxycarbonyl derivatives (*N*-acyl carbamates) degraded predominantly to the acid of the parent amide and are suggested to be less suitable than *N*-acyl derivatives as prodrug forms for the amide functionality.

Introduction

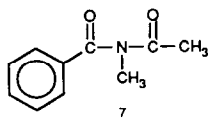
A useful approach to improve drug delivery is chemical transformation of drug substances into per se inactive derivatives (prodrugs) that convert to the parent compounds by virtue of enzymic or chemical ability within the body system (Bundgaard, 1985a, 1989; Roche, 1987; Waller and George, 1989; Balant et al., 1990). A fundamental requisite for this prodrug approach is the ready availability of chemical derivative types satisfying the prodrug requirements, the most important of these being reconversion of the prodrug to the parent drug in vivo.

A primary or secondary carboxamide moiety occurs in several drug substances as well as in many bioactive peptides, notably neural and hormonal peptides (Mains et al., 1983). To solve delivery problems associated with some of such compounds due to e.g. unfavourable lipophilicity and solubility characteristics or rapid enzymatic inactivation, by the prodrug concept, the availability of a prodrug type for the amide functionality is desirable. Previously exploited bioreversible derivatives for the amide moiety include *N*-Mannich bases and *N*-hydroxymethyl derivatives (Bundgaard, 1985b) as well as glyoxylic acid adducts

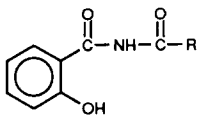
Correspondence: H. Bundgaard, The Royal Danish School of Pharmacy, Department of Pharmaceutical Chemistry, 2 Universitetsparken, DK-2100 Copenhagen, Denmark.



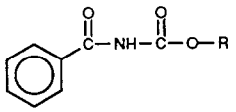
	R
1	H
2	CH_3
3	C_2H_5
4	nC_3H_7
5	nC_4H_7
6	C_6H_5

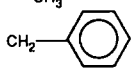
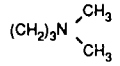


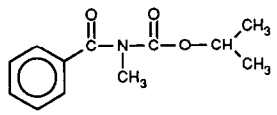
7



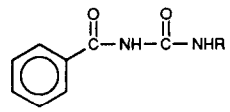
	R
8	H
9	CH_3
10	C_2H_5
11	nC_3H_7
12	C_6H_5

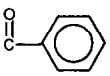


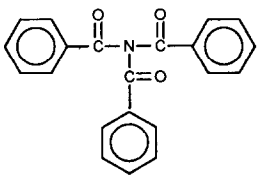
	R
13	CH_3
14	C_2H_5
15	
16	
17	
18	C_6H_5



19



	R
20	H
21	



22

(Bundgaard and Buur, 1987) and phthalidyl derivatives (Bundgaard et al., 1988a).

We have previously found that the *N*-acetyl derivative of salicylamide was relatively easily hydrolyzed enzymatically in the presence of human plasma to yield salicylamide in quantitative amounts (Bundgaard et al., 1986). This finding along with similar more recent observations with *N*-acyl derivatives of the cyclic amide 2-pyrrolidone (Bundgaard and Møss, 1989) suggested that *N*-acylation of amides should receive more attention as a prodrug approach. To explore more fully the potential usefulness of *N*-acylated amides (diacylamines) as prodrugs we have examined the kinetics of hydrolysis and plasma-catalyzed degradation of a number of *N*-acyl derivatives of benzamide, *N*-methylbenzamide and salicylamide (**1**–**19**), used as models for the carboxamide group.

Materials and Methods

Apparatus

High-performance liquid chromatography (HPLC) was performed with a Shimadzu system consisting of an LC-6A pump, an SPD-6A variable-wavelength UV detector and a 20 μl loop injection valve. A deactivated Supelcosil LC-8-DB reversed-phase column (33 × 4.6 mm) (3 μm particles) from Supelco Inc., U.S.A., was used in conjunction with a Supelguard column. In some cases a Kontron instrument consisting of an LC pump T-414, a Uvikon 740 UV detector operated at a fixed wavelength (240 or 215 nm) and a 20 μl injection valve was used. A Chrompack column (100 × 3 mm) packed with CP Spher C-8 (5 μm particles) was used with this apparatus. The pH measurements were made at the temperature of study using a Radiometer Type PHM 83 Autocal instrument.

Chemicals

Benzamide, *N*-methylbenzamide and salicylamide were obtained from Aldrich-Chemie, Germany. Chemicals and solvents used in the kinetic studies were of reagent grade.

Synthesis of N-acyl derivatives (1–19)

The *N*-acyl derivatives **2**–**5** were prepared by reacting benzamide with the appropriate acid anhydride in the presence of catalytic amounts of sulphuric acid as described by Hurd and Prapas (1959) for compound **2**. The *N*-benzoyl derivative **6** was prepared by reacting benzamide with benzoyl chloride in pyridine as reported by Lamberton and Standage (1960) whereas *N*-formylbenzamide (**1**) was prepared according to Finkbeiner (1965). All these compounds were recrystallized from ethanol or ethanol-water. They have been prepared before and their melting points were in good agreement with those reported (Table 1).

N-Acetyl-*N*-methylbenzamide (**7**) has not been described before. It was prepared by heating a mixture of *N*-methylbenzamide (1.35 g, 10 mmol), acetic anhydride (3.04 g, 20 mmol) and 100 μl of concentrated sulphuric acid at 135–140°C for 4 min. The reaction mixture was poured into 30 ml of water and extracted with ethyl acetate. The

TABLE 1

Melting points and references to reported melting points of various N-acylated amides

Compound	Melting point (°C)	Reference
1	106–107	Finkbeiner (1965)
2	116–117	Hurd and Prapas (1959)
3	96– 97	Polya and Spotswood (1948)
4	102–103	Dunn et al. (1952)
5	76– 77	Kametani et al. (1983)
6	146–147	Titherley (1904)
7	33– 34	
8	164–165	
9	140–142	Gordon (1967)
10	125–126	Hanada (1958)
11	128–129	Hanada (1958)
12	200–202	Einhorn and Schupp (1905)
13	118–119	Sheehan and Izzo (1949)
14	110–111	Neidlein and Krüll (1971)
15	99–100	Arcus and Prydal (1957)
16	113–114	Arcus and Prydal (1957)
17	86– 87	
18	103–104	Venkatasubramanian and Chanadrasekar (1982)
19	oil	
20	227–228	Speziale and Smith (1963)
21	211–212	Billeter (1903)
22	207–208	Titherley (1904)

dried extract was evaporated in vacuo and the residue obtained crystallized from ether-petroleum ether (m.p. 33–34°C). Anal.: Calc. for $C_{10}H_{11}NO_2$: C, 67.78; H, 6.26; N, 7.90. Found: C, 67.58; H, 6.26; N, 7.94.

The *N*-acyl derivatives of salicylamide (**8–12**) were prepared by refluxing the corresponding *O*-acyl salicylamide derivatives in aqueous methanol as described previously (Hanada, 1958; Bundgaard et al., 1986). The compounds had melting points in agreement with those reported (Table 1). The *N*-formyl derivative **8**, prepared from *O*-formylsalicylamide (Treppendahl and Jacobsen, 1983), has not been described before : Anal.: Calc. for $C_8H_7NO_3$: C, 58.18; H, 4.27; N, 8.48. Found: C, 58.27; H, 4.25; N, 8.42.

The *N*-alkoxycarbonyl derivatives **13–18** were prepared by reacting benzoyl isocyanate with the appropriate alcohols or phenol as described before (Arcus and Prydal, 1957; Bundgaard et al., 1988b). The benzoyl isocyanate was obtained by reacting benzamide with oxalyl chloride (Speziale et al., 1965). The compounds showed melting points in accordance with those reported (Table 1). Compound **17** is new. It was prepared by heating a solution of *N*,*N*-dimethylaminopropanol (3.51 ml, 30 mmol) and benzoyl isocyanate (30 mmol) in acetonitrile (40 ml) for 1 h at 60 °C. The solution was evaporated in vacuo and the solid residue obtained recrystallized from chloroform-ether, m.p. 86–87°C. Anal.: Calc. for $C_{13}H_{18}N_2O_3$: C, 62.38; H, 7.25; N, 11.19. Found: C, 62.23; H, 7.01; N, 11.17.

Compound **19** was prepared by methylation of compound **15**. Methyl iodide (0.3 ml, 9.6 mmol) was added to a solution of compound **15** (500 mg, 2.4 mmol) in 10 ml tetrahydrofuran followed by the addition of sodium hydride (90 mg, 3.6 mmol). The mixture was stirred at 20°C for 3 days and evaporated in vacuo. The residue obtained was taken up in ethyl acetate and the solution washed with water and evaporated in vacuo to give compound **19** as an oil. HPLC analysis showed that it contained about 10% of unreacted compound **15**.

N-Benzoylurea (**20**) and 1,3-dibenzoylurea (**21**) were obtained by reacting benzoyl isocyanate with ammonia and benzamide, respectively, as described by Billeter (1903). Tribenzamide (**22**) was prepared by treating *N*-benzoylbenzamide (**6**) with benzoyl chloride and pyridine as reported by Titherley (1904).

Kinetic measurements

All rate studies were performed in aqueous buffer solutions at 37.0 ± 0.2°C. Hydrochloric acid, acetate, phosphate, borate and carbonate buffers were used; the total buffer concentration was generally 0.02 M and a constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride.

The rates of degradation of the derivatives (**1–19**) were determined by using reversed-phase HPLC procedures capable of separating the compounds from their products of degradation. A deactivated Supelcosil column was eluted with mobile phase systems consisting of 10–50% acetonitrile in 0.1% phosphoric acid, the concentration of acetonitrile being adjusted for each compound to give an appropriate retention time (2–8 min). For product analysis a Chrompack C-8 column was eluted with 0.02 M acetate buffer of pH 4.0. The column effluent was monitored at 240 nm and the flow rate was 1.5 ml min^{-1}. Product analysis of compound **18** was performed at 215 nm with a flow rate of 1.0 ml min^{-1}. Quantitation of the compounds was done by measuring 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 derivatives in acetonitrile to 10 ml of preheated buffer solution in screw-capped test tubes, the final concentration of the compounds being 5×10^{-5}–10^{-4} M. The solutions were kept in a water-bath at 37°C and at appropriate intervals samples were taken and chromatographed immediately. 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 rate constants for the slowly proceeding reactions of compound **21** at pH 1–6 were obtained by measuring the initial rates of formation of compound **20**. The initial concentration of **21** was 10^{-4} M, and the degradation was followed up to an extent of 3% so the initial concentration can

be regarded as constant during the measurements. Pseudo-first-order rate constants for the hydrolysis were obtained by dividing the slopes of linear plots of amounts of **20** formed against time with the initial concentration of compound **21**.

The hydrolysis of the compounds was also studied in human plasma diluted to 80% with 0.05 M phosphate buffer of pH 7.4, the initial concentration of the compounds being about 10^{-4} M. At appropriate intervals samples of 250 μl were withdrawn and added to 500 μl of a 2% solution of zinc sulphate in methanol-water (1 : 1 v/v) in order to deproteinize the plasma. After mixing and centrifugation for 3 min at 13000 rpm 20 μl of the clear supernatant was analyzed by HPLC as described above.

Results and Discussion

Kinetics of hydrolysis of the N-acyl derivatives 1–12

The kinetics of hydrolytic breakdown of the *N*-acyl derivatives **1–12** were studied in aqueous solution at 37°C over a wide range of pH. At constant pH and temperature the hydrolysis displayed strict first-order kinetics for several half-lives as determined by HPLC. At the buffer concentration used (0.02 M) no significant general acid-base catalysis was observed.

The influence of pH on the overall rates of hydrolysis of the *N*-acyl derivatives **1**, **2** and **6** is shown in Fig. 1 where the logarithm of the observed pseudo-first-order rate constants (k_{obs}) is plotted against pH. The effect of pH upon the hydrolysis of *N*-acetylbenzamide (**2**) was examined in detail in the pH range 1.1–10.8. The U-shape of the pH-rate profile indicates the occurrence of specific acid and base catalysis as well as a spontaneous or water-catalyzed reaction. In the case of *N*-benzoyl benzamide (**6**), however, the rate of hydrolysis does not continue to increase with increasing pH but levels off at pH > 9. At this pH the NH proton begins to dissociate and the leveling off of the rate of hydrolysis is thus indicative of the non-reactivity of the ionized *N*-acylated amide (Scheme 1) as has also been reported for the alkaline hydrolysis of other *N*-acyl amides (Titherley and Stubbs, 1914; Edward and Terry, 1957; Lamberton and Standage, 1960; Behme and Cordes, 1964) and cyclic imides and hydantoins (Edward and Terry, 1957; Yakatan and Fan, 1977; Blagoeva et al., 1978). Due to the greater electronegativity of the benzoyl group relative to the alkyl groups in compounds **2–5** the pK_a value of compound **6** is lower than that for the other compounds. Therefore, the curvature in the pH-rate profile was only observed for compound **6** in the pH range studied. The rate expression for the hydrolysis of the derivatives may accordingly be:

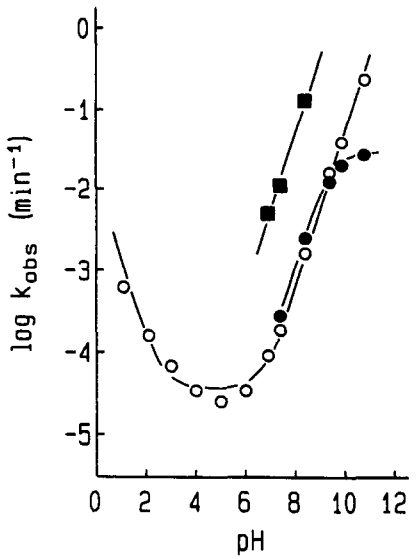
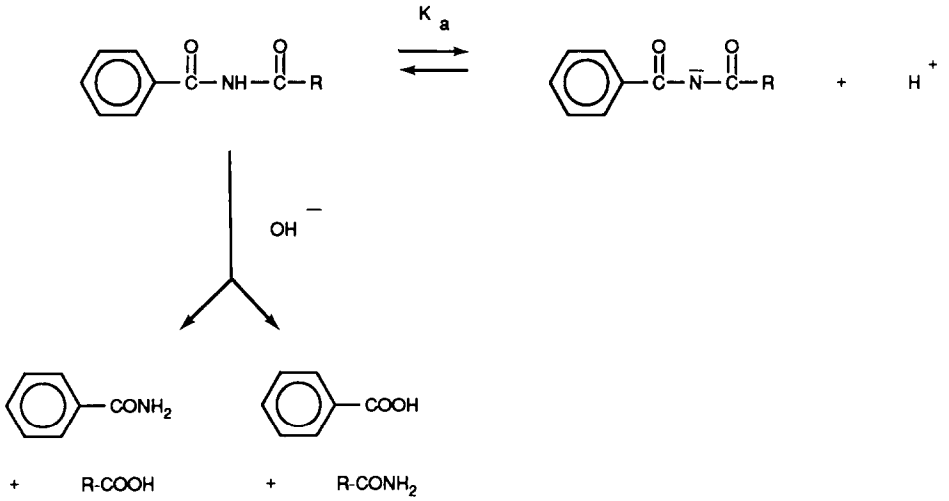


Fig. 1. The pH-rate profiles for the hydrolysis of the *N*-acyl derivatives **1** (■), **2** (○) and **6** (●) in aqueous solution at 37°C ($\mu = 0.5$).

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

where k_0 is a first-order rate constant for spontaneous or water-catalyzed degradation of the undissociated species, k_H and k_{OH} are second-order rate constants for the specific acid- and base-catalyzed hydrolysis of the undissociated species, respectively, $a_H/(a_H + K_a)$ is the fraction of this species, K_a is the ionization constant of the NH moiety and a_H and a_{OH} are the hydrogen and hydroxide ion activities, respectively. The latter



Scheme 1.

was calculated from the pH at 37°C according to the following equation (Harned and Hamer, 1933):

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

Values of the specific rate constants k_H and k_{OH} were determined from the straight line portions of the profile at low and high pH values, respectively, whereas the value of k_0 was obtained from Eqn 1. For compound **2** the following values were obtained:

$$k_H = 1.2 \times 10^{-2}\ M^{-1}\ min^{-1}$$

$$k_{OH} = 2.0 \times 10^{2}\ M^{-1}\ min^{-1}$$

$$k_0 = 3.5 \times 10^{-5}\ min^{-1}$$

Rate data for compounds **1**–**7** are shown in Table 2.

The hydrolysis of the *N*-acyl amides in neutral and alkaline solutions may proceed by attack of hydroxide ions on both of the carbonyl groups as depicted in Scheme 1. HPLC analysis of the reaction solutions showed that whereas the most reactive compound, *N*-formylbenzamide (**1**), only hydrolyzed to produce benzamide, the other unsymmetrical *N*-acyl amides **2**–**5** hydrolyzed by concurrent attack of hydroxide ions at the two carbonyl groups. For these compounds benzoic acid was formed in approx. 30% yield and benzamide in about 70% yield. The hydrolysis of the symmetrical diacylamine **6** produced both benzamide and benzoic acid in the expected 100% yield. The hydrolysis of the *N*-methylated diacylamine **7** proceeded with formation of 38% benzamide and 62% benzoic acid, indicating that the *N*-methyl group favours hydroxide ion attack on the benzoyl carbonyl group. As previously discussed by Lamberton and Standage (1960) both steric and polar effects within the acyl groups of diacylamines determine the relative rates of attack on the dissimilar carbonyl groups.

The hydrolytic behaviour of various *N*-acyl de-

TABLE 2

Rate data and half-lives for the hydrolysis of various N-acyl derivatives of benzamide and N-methylbenzamide in aqueous solution and in 80% human plasma (pH 7.4) at 37°C

Compound	k_{OH} ($M^{-1}\ min^{-1}$)	Half-lives (h)	
		pH 7.4 buffer	80% plasma
1	2.0×10^4	1.1	0.25
2	200	61	6.4
3	271	48	9.2
4	220	61	8.9
5	234	53	6.9
6	480	41	2.5
7	295	42	6.7

rivatives of salicylamide (**8–12**) was examined in buffer solutions of pH 7.4–10.8 at 37°C. The pH-rate profiles for compounds **8**, **9** and **11** are shown in Fig. 2. In the pH range investigated the degradation can be described by the following rate expression:

$$k_{obs} = k_{OH} a_{OH} (a_H/(a_H + K_a) + k'_{OH} a_{OH} (K_a/(a_H + K_a) \quad (3)$$

where K_a is the apparent ionization constant for the phenol group and k_{OH} and k'_{OH} are second-order rate constants for the hydroxide ion-catalyzed hydrolysis of the undissociated and ionized species, respectively (Scheme 2). The values of the rate constants derived along with the kinetically determined pK_a values are listed in Table 3.

Inspection of the rate data shows that ionization of the phenolic group makes the diacylamines about 100-fold more resistant to hydrolysis. This may be a result of electrostatic repulsion by the phenolate ion of hydroxide ion attack. When the phenolic group is undissociated the stability of the compounds (k_{OH} values) is almost similar to that of the corresponding benzamide derivatives. The salicylamide derivatives differed, however, from the benzamide analogs in that the compounds only hydrolyzed at the carbonyl group remote from the salicylamide moiety, i.e. the hydrolysis of **8–12** proceeded with quantitative formation of salicylamide at pH 7.4–10.8. Even traces of salicylic acid were not detected by HPLC analysis. This behaviour may be ascribed to the steric hindrance exhibited by the *ortho*-situated hydroxyl group. It is interesting to note that while an intramolecular general base-catalytic effect of the ionized phenol group has been shown to be involved in the hydrolysis of salicylamide (Bruice and Tanner, 1965), such effect is apparently not

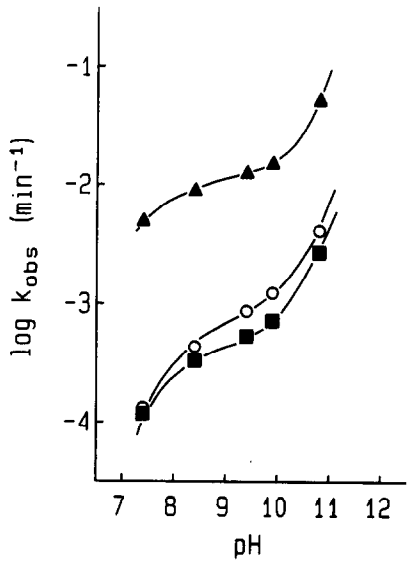
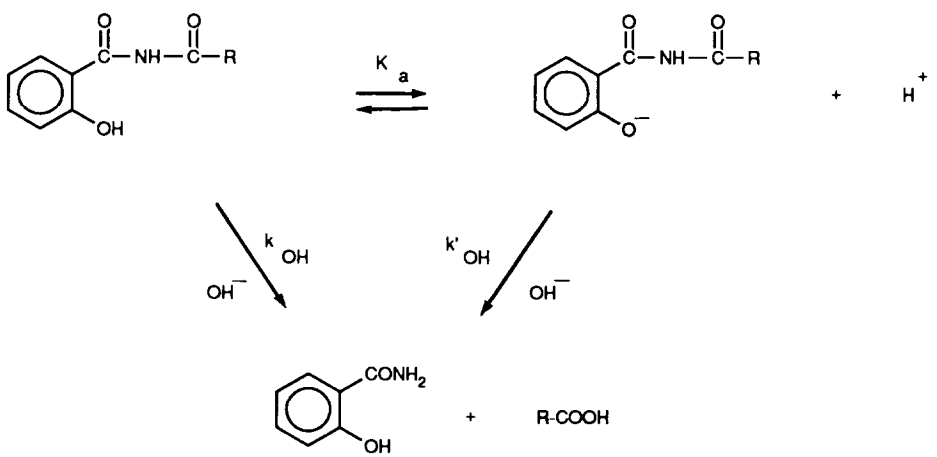


Fig. 2. The pH-rate profiles for the hydrolysis of the *N*-acyl derivatives **8** (▲), **9** (○) and **11** (■) in aqueous solution at 37°C ($\mu = 0.5$).



Scheme 2.

TABLE 3

Rate data and half-lives for the hydrolysis of various N-acyl derivatives of salicylamide in aqueous solution and in 80% human plasma (pH 7.4) at 37°C

Compound	k_{OH} (M^{-1} min^{-1})	k'_{OH} (M^{-1} min^{-1})	pK_a	Half-lives (h)	
				pH 7.4 buffer	80% plasma
8	7.5×10^3	19	7.8	2.9	2.1
9	202	2.7	8.1	88	4.5
10	265	2.6	8.0	73	3.8
11	225	1.7	7.9	98	5.0
12	220	0.9	7.6	120	11

operating for the *N*-acyl salicylamides. If this was the case the salicylamide derivatives should be more reactive than the corresponding benzamide derivatives at pH 7–8.

*Enzymatic hydrolysis of the N-acyl derivatives **1–12***

The susceptibility of the *N*-acyl derivatives of benzamide, *N*-methylbenzamide and salicylamide to undergo a potential enzymatic hydrolysis was studied at 37°C in human plasma diluted to 80% with 0.05 M phosphate buffer of pH 7.4. The hydrolysis of the derivatives followed strict first-order kinetics (Fig. 3). From the rate data obtained (Tables 2 and 3) it is seen that plasma enzymes markedly catalyze the rate of hydrolysis.

HPLC analysis of completed reaction solutions showed that the plasma-catalyzed hydrolysis occurred predominantly at the alkyl amide bond. Thus, benzamide was produced in quantitative amounts from compounds **1**, **4** and **5** (and **6**) whereas it was formed to an extent of 80 and 86% from compounds **2** and **3**, respectively. For compound **7**, the *N*-methylbenzamide was formed in an amount of 66%. In the case of the *N*-acyl salicylamide derivatives the parent salicylamide was produced in quantitative amounts as in buffer solutions. In accordance with the facile plasma-catalyzed hydrolysis of the *N*-acyl amide derivatives is the finding several years ago that *N*-acetylsalicylamide is completely transformed to salicylamide and metabolites thereof in man after oral administration (Rayet et al., 1951).

Kinetics of hydrolysis of N-alkoxycarbonyl derivatives

The degradation of various *N*-alkoxycarbonyl derivatives of benzamide (**13–18**) and *N*-methylbenzamide (**19**) was studied in aqueous buffer solutions at 37°C. Some rate data appear from Fig. 4 and Table 4. The hydrolysis of compound

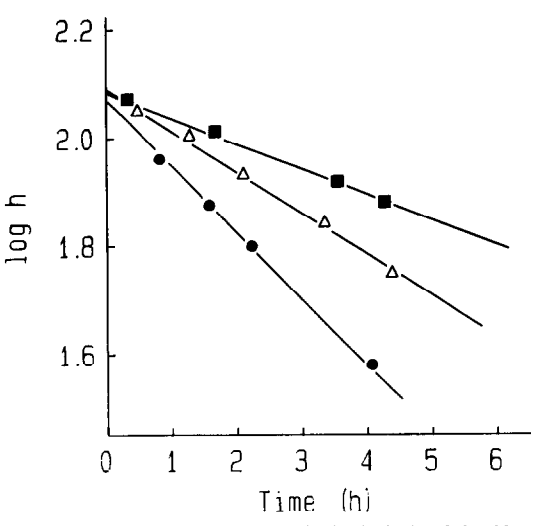


Fig. 3. First-order kinetic plots for the hydrolysis of the *N*-acyl derivatives **6** (●), **7** (■) and **10** (△) in 80% human plasma (pH 7.4) at 37°C.

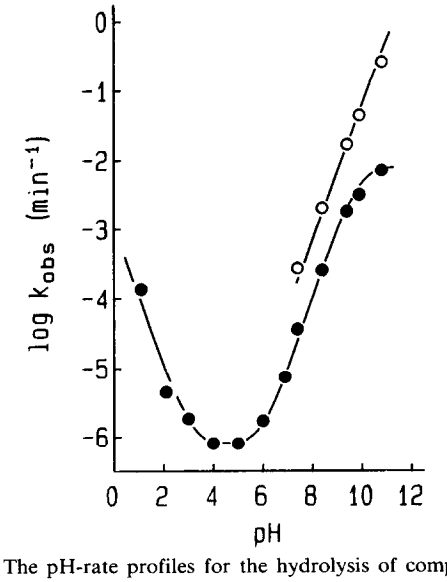


Fig. 4. The pH-rate profiles for the hydrolysis of compounds **13** (●) and **19** (○) in aqueous solution at 37°C ($\mu = 0.5$).

TABLE 4

Rate data and half-lives for the hydrolysis of various N-alkoxycarbonyl derivatives of benzamide and N-methylbenzamide in aqueous solution and in 80% human plasma (pH 7.4) at 37 °C

Compound	k_{OH} (M^{-1} min^{-1})	pK_a	Half-lives (h)	
			pH 7.4 buffer	80% plasma
13	40	9.9	318 h	43 h
14	10 [a]	10.4 [a]	963 h [b]	16.2
15	17	10.1	816 h [b]	
16	32	10.0	482 h [b]	
17	300	9.5	75 h	7.3 h
18		8.9 [a]	10 min	5 sec
19	30		514 h [b]	25 h

[a] Data from Bundgaard et al. (1988b).
[b] Calculated on the basis of the k_{OH} values.

13 was studied over a broad pH range and the pH-rate profile obtained is shown in Fig. 4. It can be accounted for by Eqn 1 with the following rate parameters:

$$k_H = 1.0 \times 10^{-3}\ M^{-1}\ min^{-1}$$

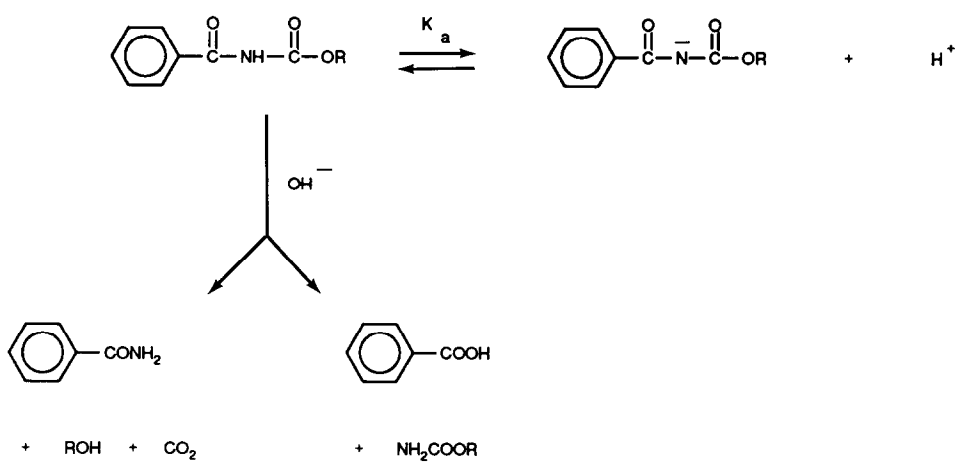
$$k_0 = 7 \times 10^{-7}\ min^{-1}$$

$$k_{OH} = 40\ M^{-1}\ min^{-1}$$

$$pK_a = 9.9$$

It has previously been established that the alkaline hydrolysis of *N*-alkoxycarbonyl amides (*N*-acyl carbamates) derived from alcohols occurs by the $B_{AC}2$ pathway with nucleophilic attack at the carbonyl carbon atom of the non-ionized substrate (Scheme 3) (Bergon and Calmon, 1976; Bundgaard et a., 1988b). The present data are in harmony with and further supportive of this mechanism. As can be seen from Fig. 4 the rate of hydrolysis of the *N*-methyl derivative **19**, which is unable to ionize, does not level off at high pH in contrast to compound **15**. This excludes the occurrence of a water-catalyzed reaction of the ionized species of compound **13** and analogs. Such a reaction is kinetically equivalent to a reaction involving hydroxide ion attack on the undissociated form.

Previous studies have indicated that the hydrolysis of *N*-acyl carbamates only occurs at the carbamate bond (Bergon and Calmon, 1976; Bundgaard et a., 1988b). HPLC analysis of completed reaction solutions of the compounds **13**–**17** showed, however, that this is not the case. As for the *N*-acyl benzamides the hydrolysis occurs concomitantly at both the carbonyl groups as depicted in Scheme 3. Thus, in solutions of pH > 7 benzoic acid was produced in amounts of 49% (**13**), 76% (**14**), 87% (**15**), 64% (**16**), 57% (**17**) and 100% (**19**). The sum of benzoic acid and benzamide formed corresponded in all cases to 100 ±



Scheme 3.

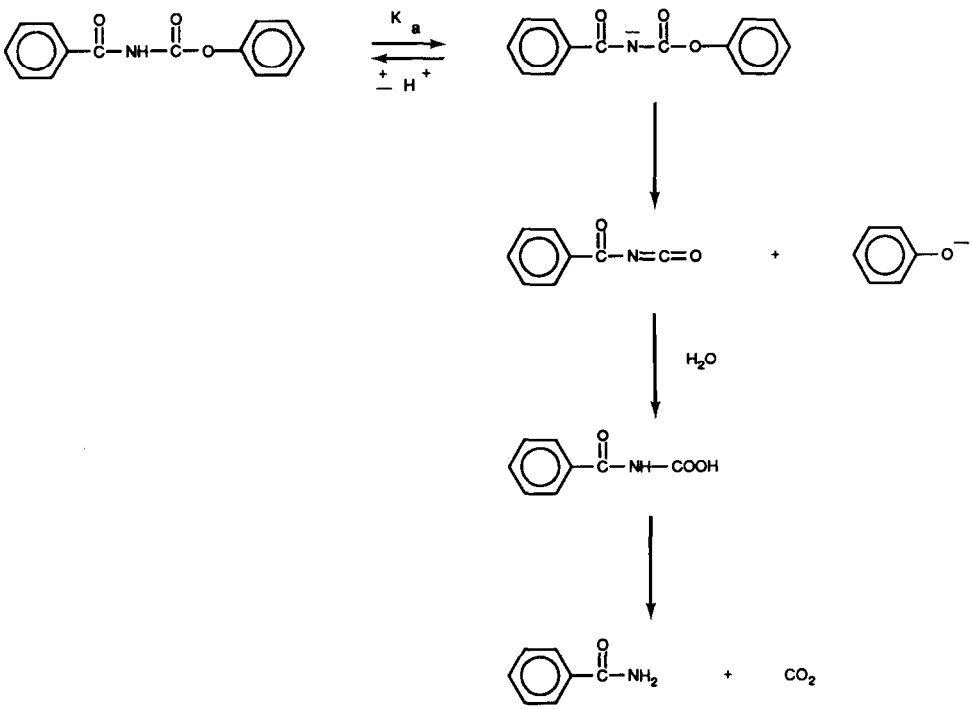
5%. As can be seen the predominant degradation route is in fact cleavage of the *N*-acyl carbamate bond except for compound **13** where the two routes are equally important.

The *N*-alkoxycarbonyl derivatives were subject to plasma-catalyzed hydrolysis as seen from the data in Table 4. Also in plasma solutions the predominant degradation product was benzoic acid, it being formed to an extent of 82% from both compounds **13** and **14** and 100% from compound **19**.

In contrast to these *N*-acyl carbamates derived from alcohols the *N*-benzoyl carbamate ester of phenol (**18**) degraded exclusively to benzamide (and phenol) as shown by HPLC analysis. As reported previously (Bergon and Calmon, 1976; Moravcova and Vecera, 1977; Venkatasubramanian and Chandrasekar, 1982) an E1cB elimination mechanism involving an unstable isocyanate intermediate (Scheme 4) is involved for *N*-acyl carbamates of phenols. At pH 7.4 and 37°C the half-life of decomposition of compound **18** is only 10 min. In the presence of human plasma the half-life dropped to 5 s (Table 4). Interestingly, although phenol was found to be formed in 100% yield in 80% plasma solutions no benzamide could be detected by HPLC. Plasma proteins are apparently being carbamoylated by compound **18** either by a direct reaction of the compound with e.g. free amino groups in the proteins or via benzoyl isocyanate.

Hydrolysis of other derivatives

Besides the *N*-acyl and *N*-alkoxycarbonyl derivatives described above the potential prodrug behaviour of the diacyl urea derivative **21** and tribenzamide (**22**) was examined. The pH-rate profile for the hydrolysis of compound **21** at 37°C is shown in Fig. 5. The reactions taking place can be



Scheme 4.

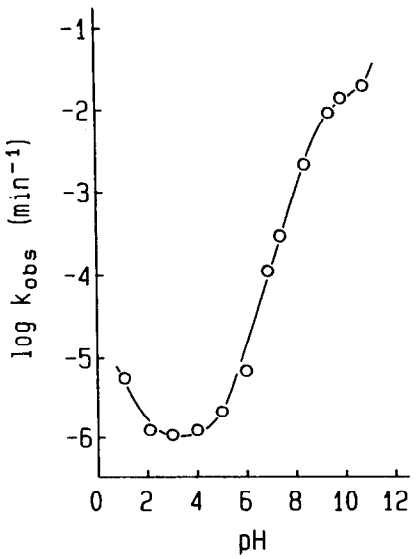


Fig. 5. The pH-rate profile for the hydrolysis of compound **21** in aqueous solution at 37°C ($\mu = 0.5$).

described by Eqn 1 using the following rate and pK_a parameters:

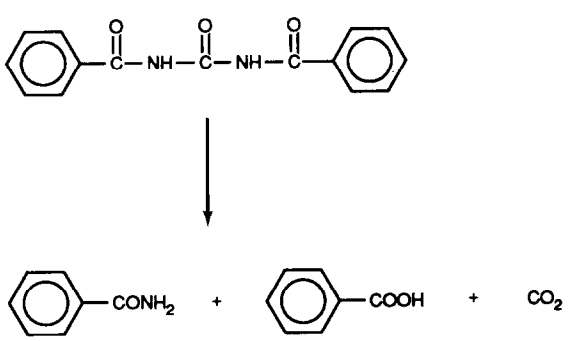
$k_H = 5.3 \times 10^{-5}$ M^{-1} min^{-1}

$k_0 = 9.8 \times 10^{-7}$ min^{-1}

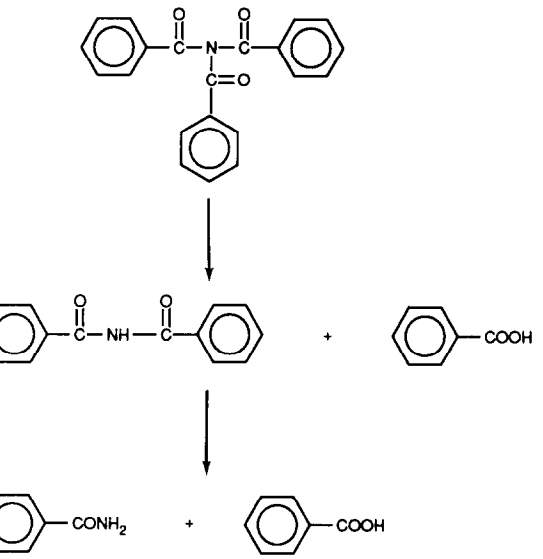
$k_{OH} = 490$ M^{-1} min^{-1}

p$K_a = 9.1$

As revealed by HPLC analysis 1,3-dibenzoylurea (**21**) degraded quantitatively to benzoic acid and *N*-benzoylurea (**20**) (Scheme 5). At pH 7.4



Scheme 5.



Scheme 6.

and 37°C the half-life of hydrolysis was 39 h. The hydrolysis was not catalyzed by human plasma.

Tribenzamide (**22**) degraded in alkaline solutions according to Scheme 6 with *N*-benzoylbenzamide as an intermediate (Fig. 6). The k_{OH} value for compound **22** was determined to be 100 M^{-1} min^{-1} in 20% acetonitrile solutions. Acetonitrile was added to the solutions because of the very low water solubility of the compound. Comparing this k_{OH} value with that for *N*-benzoylbenzamide (**6**) (480 M^{-1} min^{-1}) shows that the

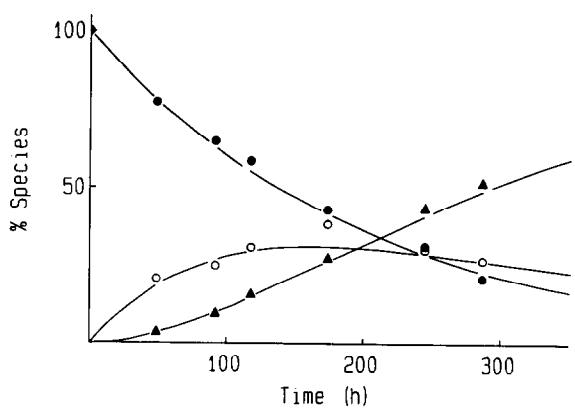


Fig. 6. Plots showing the time courses of degradation of compound **22** (●) and formation of compound **21** (○) and benzamide (▲) in aqueous buffer solution of pH 10.1 containing 20% acetonitrile (37°C).

diacylated amide (**22**) is less reactive than the corresponding monoacylated amide. The presence of acetonitrile may, however, lower the rate of hydrolysis somewhat.

Conclusions

The results described show that *N*-acylation of primary or secondary amides may be a useful prodrug approach. *N*-Acylated amides (diacylamines) are relatively stable in aqueous solution but readily hydrolyzed enzymatically by e.g. human plasma. For a given amide-containing drug, however, the usefulness of this approach depends on a close examination of the route of cleavage of the *N*-acylated amides since the compounds can be hydrolyzed both chemically and enzymatically to either the parent amide or the corresponding carboxylic acid. *N*-Alkyloxycarbonylation appears a less suitable approach since *N*-alkyloxycarbonyl derivatives are mainly cleaved to the acid of the parent amide as determined with benzamide derivatives. The possible utility of the *N*-acylation prodrug approach to protect the C-terminal amide group of various peptides against cleavage by proteolytic enzymes such as α-chymotrypsin is presently being examined in this laboratory.

References

Arcus, C.L. and Prydal, B.S., Isocyanates. III. Benzoyl isocyanate. *J. Chem. Soc.*, (1957) 1091–1093.

Balant, L.P., Doelker, E. and Buri, P., Prodrugs for the improvement of drug absorption via different routes of administration. *Eur. J. Drug Metab. Pharmacokinet.*, 15 (1990) 143–153.

Behme, M.T. and Cordes, E.H., Intramolecular participation of the amide group in ester hydrolysis. *J. Org. Chem.*, 29 (1964) 1255–1257.

Bergon, M. and Calmon, J.-P., Hydrolyse alcaline de *N*-acétylcarbamates. Changement de mécanisme lié a la nature du groupe ester. *Bull. Soc. Chim. Fr.*, (1976) 797–802.

Biletter, O.C., Ueber die Einwirkung von cyansäurem Silber auf Säurechloride. II. Benzoylisocyanate. *Chem. Ber.*, 36 (1903) 3218–3221.

Blagoeva, I.B., Pojarlieff, I.G. and Dimitrov, V.S., Alkaline hydrolysis of hydantoin, 3-methylhydantoin, and 1-acetyl-3-methylurea. Effect of ring size on the cleavage of acylureas. *J. Chem. Soc. Perkin Trans. II*, (1978) 887–892.

Bruice, T.C. and Tanner, D.W., Neighboring hydroxyl group assistance. VI. In amide hydrolysis. *J. Org. Chem.*, 30 (1965) 1668–1669.

Bundgaard, H., *Design of Prodrugs*, Elsevier, Amsterdam, 1985a.

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

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

Bundgaard, H. and Buur, A., 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. *Int. J. Pharm.*, 37 (1987) 185–194.

Bundgaard, H., Buur, A., Hansen, K.T., Larsen, J.D., Møss, J. and Olsen, L., Prodrugs as drug delivery systems. 77. Phthalidyl derivatives as prodrug forms for amides, sulfonamides, carbamates and other NH-acidic compounds. *Int. J. Pharm.*, 45 (1988a) 47–57.

Bundgaard, H., Buur, A. and Lee, V.H.L., Timolol prodrugs: Preparation and hydrolysis kinetics of N-benzoyl carbamate esters of timolol and related compounds. *Acta Pharm. Suec.*, 25 (1988b) 293–306.

Bundgaard, H., Klixbüll, U. and Falch, E., Prodrugs as drug delivery systems. 44. *O*-acyloxymethyl, *O*-acyl and *N*-acyl salicylamide derivatives as possible prodrugs for salicylamide. *Int. J. Pharm.*, 30 (1986) 111–121.

Bundgaard, H. and Møss, J. Prodrugs of peptides. IV. Bioreversible derivatization of the pyroglutamyl group by *N*-acylation and *N*-aminomethylation to effect protection against pyroglutamyl aminopeptidase. *J. Pharm. Sci.*, 78 (1989) 122–126.

Dunn, P., Parkes, E.A. and Polya, J.B., Amides. IX. Acetylation of amides and amines. *Rec. Trav. Chim. Pays-Bas*, 71 (1952) 676–683.

Edward, J.T. and Terry, K.A., The hydrolysis of amides and related compounds. Part IV. Diacetylamine and succinimide in aqueous alkali. *J. Chem. Soc.*, (1957) 3527–3530.

Einhorn, A. and Schupp, G., Ueber Benzoylirung des Salicylamids. *Chem. Ber.*, 38 (1905) 2792–2798.

Finkbeiner, H., A preparation of aroyl and acyl formamidinium salts. *J. Org. Chem.*, 30 (1965) 2861–2862.

Gordon, A.J., Acetylsalicylamide. *O*- to *N*-acetyl migration. *Tetrahedron*, 23 (1967) 863–870.

Hanada, T., Synthesis of 2-alkyl-4H,1,3-benzoxazine-4-ones. *Bull. Chem. Soc. Jap.*, 31 (1958) 1024–1026.

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.

Hurd, C.D. and Prapas, A.G., Preparation of acyclic imides. *J. Org. Chem.*, 24 (1959) 388–392.

Kametani, T., Kigasawa, K., Shimizu, H., Saitou, S., Hayashida,

S. and Fujino, M., Transacylation between phenyl acylates and amides. II. Transacylation between *p*-nitrophenyl acylates and benzamides. *J. Pharm. Soc. Jap.*, 103 (1983) 1144–1150.

Lamberton, A.H. and Standage, A.E., The alkaline hydrolysis of some *N*-acyl-benzanilides and -benzamides, with a note on the behaviour of *N*-acylbenzanilides in sulphuric acid. *J. Chem. Soc.*, (1960) 2957–2966.

Mains, R.E., Eipper, B.A., Glembotski, C.C. and Dores, R.M., Strategies for the biosynthesis of bioactive peptides. *Trends Neurosci.*, 6 (1983) 229–235.

Moravcova, J. and Vecera, M., Kinetics and mechanism of hydrolysis of aryl *N*-trichloroacetylcarbamates. *Coll. Czech. Chem. Commun.*, 42 (1977) 3048–3056.

Neidlein, R. and Krüll, H., Zum Reaktionsverhalten acylierter Carbamidsäureazide gegenüber Diazomethan. *Arch. Pharm.*, 304 (1971) 749–753.

Polya, J.B. and Spotswood, T.M., Amides. II. Acylation of amides to diacylimines. *Rec. Trav. Chim. Pays-Bas*, 67 (1948) 927–941.

Rayet, P., Baloueff, S., Bruckner, P. and Philippot, E., Métabolisme de la N-acétylsalicylamide. Elimination urinaire de la N-acétylsalicylamide chez l'homme normal. *Arch. Int. Pharmacodyn.*, 88 (1951) 159–202.

Roche, E.B., *Bioreversible Carriers in Drug Design: Theory and Application*, Pergamon, New York, 1987.

Sheehan, J.C. and Izzo, P.T., The reaction of diazomethane with isocyanates and isothiocyanates. *J. Am. Chem. Soc.*, 71 (1949) 4059–4062.

Speziale, A.J. and Smith, L.R., The reaction of oxalyl chloride with amides. II. Oxazolidinediones and acyl isocyanates. *J. Org. Chem.*, 28 (1963) 1805–1811.

Speziale, A.J., Smith, L.R. and Fedder, J.E., The reaction of oxalyl chloride with amides. IV. Synthesis of acyl isocyanates. *J. Org. Chem.*, 30 (1965) 4306–4307.

Titherley, A.W., The acylation of amides. *J. Chem. Soc.*, 85 (1904) 1673–1692.

Titherley, A.W. and Stubbs, L., The hydrolysis of mixed secondary amides by alkalis. *J. Chem. Soc.*, 105 (1914) 299–309.

Treppendahl, S. and Jacobsen, P. The reaction between acetic formic anhydride and salicylamide. *Acta Chem. Scand.*, B 37 (1983) 953–958.

Venkatasubramanian, N. and Chandrasekar, R., Structural and solvent influences on the alkaline hydrolysis of *N*-acyl carbamates. In Kehl, H. (Ed.), *Chemistry and Biology of Hydroxamic Acids*, Karger, Basel, 1982, pp. 63–71.

Waller, D.G. and George, C.F., Prodrugs. *Br. J. Clin. Pharmacol.*, 28 (1989) 497–507.

Yakatan, G.J. and Fan, T., Kinetics of hydrolysis of succinimides. *Drug Dev. Ind. Pharm.*, 3 (1977) 315–338.