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Prodrugs as drug delivery systems. 68. Chemical and plasma-catalyzed hydrolysis of various esters of benzoic acid: a reference system for designing prodrug esters of carboxylic acid agents

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Summary

The hydrolysis of a series of esters of benzoic acid including various glycolic acid derivatives was studied in alkaline solution and in human plasma at 37 °C. For the hydroxide ion-catalyzed hydrolysis a linear free-energy relationship between log k_{OH} and the Taft polar parameter σ^* for the substituents in the alcohol portion was derived. The linear correlation equation obtained covered esters with a 100-fold variation in reactivity. All esters hydrolyzed to give benzoic acid except for various benzoylglycolic acid esters which predominantly hydrolyzed to give benzoylglycolic acid. The susceptibility of the ester derivatives to undergo enzyme-catalyzed hydrolysis by human plasma was strongly influenced by the structure of the alcohol moiety and was unrelated to the chemical reactivity of the compounds. Among the alkyl esters the ethyl ester showed the least enzymatic lability whereas the choline, *N*,*N*-dimethylaminoethanol and *N*,*N*-dimethylglycolamide esters were hydrolyzed extremely rapidly. The benzoylglycolic acid esters were predominantly, although incompletely, cleaved to benzoic acid by plasma enzymes. The results obtained were discussed in relation to design of ester prodrugs of carboxylic acid agents.

Introduction

In recent years considerable attention has been focused on the use of bioreversible derivatives (prodrugs) in order to improve the delivery characteristics of various drugs (Stella, 1975; Bundgaard, 1985). A basal requisite for the usefulness of the prodrug approach is the ready 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. Several types of bioreversible derivatives for various functional groups and chemical entities have been exploited for utilization in designing prodrugs and it is now feasible to obtain prodrug derivatives of many different drug molecules (Bundgaard, 1985).

There is, however, still a great need to explore new prodrug types, even in the field of esters. Such derivatives are probably the best known prodrugs, a major reason for this being the pre-

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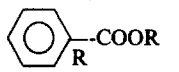
dominance of carboxylic or hydroxyl groups in drug molecules along with the ready availability of enzymes in the organism capable of hydrolyzing most esters. The distribution of esterases is ubiquitous, and several types can be found in the blood, liver and other organs or tissues (La Du and Snady, 1971). Several examples of ester prodrugs can be found in various reviews (Digenis and Swintosky, 1975; Morozowich et al., 1977; Sinkula and Yalkowsky, 1975; Bundgaard, 1985). Sometimes, however, many aliphatic or aromatic esters are not sufficiently labile in vivo to ensure a sufficiently high rate and extent of prodrug conversion. For example, simple alkyl and aryl esters of penicillins are not hydrolyzed to the active free penicillin acid in vivo (Holysz and Stavely, 1950; Ferres, 1983) and therefore have no therapeutic potential. Similarly, methyl and ethyl esters of naproxen, indomethacin and other related carboxylic acid drugs are only slowly hydrolyzed in human plasma (Bundgaard and Nielsen, 1987). In the field of angiotensin-converting enzyme inhibitors ethyl esters have been developed as prodrugs for the parent active carboxylic acid drugs in order to improve the oral bioavailability of these hydrophilic agents (Cohen, 1985), but the limited susceptibility of these esters to undergo enzymatic hydrolysis in vivo has been shown to result in incomplete availability of the active parent acid in a number of cases including enalapril (Tocco et al., 1982; Larmour et al., 1985; Todd and Heel, 1986; Rakhit and Tipnis, 1984; Rakhit et al., 1985; Hajdú et al., 1984).

In attempting to explore new generally applicable ester prodrug types possessing a high susceptibility to undergo enzymatic hydrolysis in plasma or blood, we recently discovered that esters of certain 2-hydroxyacetamides (glycolamides) are cleaved extremely rapidly in human plasma and may have great utility as a prodrug type for drugs containing a carboxylic acid function (Bundgaard and Nielsen, 1987). To fully evaluate the enzymatic lability of these esters in relation to that of other esters of the same acid and in order to expand the utility of esterification as a means of obtaining prodrug forms of carboxylic acid agents, more information is needed about the chemical and enzymatic lability of a broad spectrum of esters of the same acid. To provide such information we have investigated the chemical- and enzyme-mediated hydrolysis of a great number of esters (Table 1) using benzoic acid as a model of a carboxylic acid drug. Human plasma was used as enzyme source instead of various specific enzyme preparations in order to have conditions simulating those occurring in vivo. It is thought that the data obtained on the influence of the chemical structure of the alcohol part on ester hydrolysis may be useful in the design of suitable prodrugs for drugs with a carboxylic acid functional group.

TABLE 1

Chemical structures of various esters of benzoic acid investigated in this study

Compound	
1	$-CH_3$
2	$-CH_2CH_3$
3	$-CH_2CH_2CH_3$
4	$-CH_2CH_2CH_2CH_3$
5	$-CH_2CH_2CH_2CH_2CH_3$
6	$-CH_2-$ 
7	$-CH_2CH_2-$ 
8	$-CH_2COOH$
9	$-CH_2COOCH_3$
10	$-CH_2COOCH_2CH_3$
11	$-CH_2COOCH_2-$ 
12	$-CH_2CONH_2$
13	$-CH_2CON(CH_3)_2$
14	$-CH_2SCH_3$
15	$-CH_2SOCH_3$
16	$-CH_2SO_2CH_3$
17	$-CH_2CH_2N(CH_3)_2,HCl$
18	$-CH_2CH_2N(CH_3)_3^+,Cl^-$
19	$-CH_2NHCO-$ 

Materials and Methods

Apparatus

Ultraviolet spectral measurements were per-

formed with a Shimadzu UV-190 spectrophotometer equipped with a thermostated cell compartment, using 1-cm quartz cuvettes. Melting points were taken on a capillary melting-point apparatus and are uncorrected. High-performance liquid chromatography (HPLC) was generally done with a Spectra-Physics Model 3500 B 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. In some cases a HPLC-system consisting of.a Kontron T-414 LC pump, a Kontron Uvikon 740 LC detector operated at a fixed wavelength (215 nm) and a Rheodyne 7125 injection valve with a 20 μl loop and a Chrompack column (100 × 3 mm) packed with Chromospher C8 (5 μm particles) was used.

Synthesis of benzoate esters

Methyl-, ethyl-, propyl-, butyl-, amyl-, benzyl- and phenyl-ethyl benzoate (compounds 1–7) and benzoylcholine chloride (compound 18) were purchased from E. Merck, F.R.G. or A.G. Fluka, Switzerland.

2-(Benzoyloxy)acetamide (12) was prepared according to Concilio and Bongini (1966) by heating an aqueous solution (150 ml) of sodium benzoate (28.8 g, 0.2 mol), 2-chloroacetamide (18.7 g, 0.2 mol) and sodium iodide (7.5 g) for 6 h at 90 °C. The title compound precipitated upon cooling, m.p. 120.5–121 °C (from ethanol–water), rep. m.p. 121–122 °C (Boudreau and Williams, 1977).

Benzoylglycolic acid (8) was prepared by acidic hydrolysis of compound 12 according to Concilio and Bongini (1966), m.p. 111–112 °C, rep. m.p. 111–112 °C.

2-(Benzoyloxy)-N,N-dimethylacetamide (13). Benzoic acid (2.44 g, 0.02 mol) and 2-chloro-*N,N*-dimethylacetamide (2.43 g, 0.02 mol) were dissolved in 10 ml of *N,N*-dimethylformamide. Sodium iodide (300 mg, 2 mmol) and triethylamine (2.02 g, 0.02 mol) were added and the mixture was stirred at room temperature (20–25 °C) overnight. After addition of 50 ml of water the reaction mixture was extracted twice with ethyl acetate. The combined extracts were washed with a 1% solution of sodium thiosulphate, a 2% aqueous solution of sodium bicarbonate and water. After drying over anhydrous sodium sulphate the solution was evaporated in vacuo. The residue obtained was recrystallized from ethanol–water to give 3.5 g (85%) of the title compound, m.p. 81–82 °C. Anal.: Calc. for $C_{11}H_{13}NO_3$: C, 63.75; H, 6.32; N, 6.76. Found: C, 63.67; H, 6.33; N, 6.75.

The *esters 9–11* were prepared by reacting benzoic acid and the corresponding alkyl bromoacetate as described above for compound 13, omitting the addition of sodium iodide. The physical data (b.p. or m.p.) of the compounds agreed with literature data (Ringshaw and Smith, 1964; Micheel and Haarhoff, 1940).

The *esters 14–16* were prepared as described by Loftsson et al. (1981) whereas *compound 17* (HCl salt), was prepared by reacting benzoyl chloride with *N,N*-dimethylaminoethanol as described by David and Ross (1950). *N-(Benzoyloxymethyl)benzamide (19)* was obtained by reacting sodium benzoate with *N*-chloromethylbenzamide in tetrahydrofuran as described by Böhme and Tippmann (1976).

Kinetic measurements

All studies were performed at 37.0 ± 0.2 °C. The alkaline hydrolysis of the esters 1–18 was studied in aqueous sodium hydroxide buffer solutions in the concentration range 0.001–0.22 M NaOH. A constant ionic strength (μ) of 0.5 was maintained for each solution by adding a calculated amount of potassium chloride. For compounds 1–8 and 12–18 the progress of the reactions was followed by direct UV-spectrophotometry. The reactions were performed in 2.5 ml aliquot portions of buffer solutions in a thermostated quartz cuvette and were initiated by adding 25 μl of stock solutions of the derivatives in acetonitrile (or water for compounds 17 and 18) to give a final concentration of about 1×10^{-4} M. The rate of hydrolysis of the compounds was followed by monitoring the decrease in absorbance at 235 nm. Pseudo-first-order rate constants were determined from the slopes of linear plots of log $(A_t - A_\infty)$ vs. time, where A_t and A_∞ are the absorbance readings at time t and infinity, respectively.

The alkaline hydrolysis of compounds 9–11 and the hydrolysis of all esters in 80% human

plasma at pH 7.4 and 37 °C was followed using reversed-phase HPLC procedures.

Mobile phase systems of 40–65% v/v methanol in 0.02 M acetate buffer of pH 4.5 were generally used, the concentration of methanol being adjusted for each compound to give appropriate retention times (2–5 min). For product analysis of benzoic acid formed and, in some cases, benzoylglycolic acid, a mobile phase system consisting of 50% methanol in the acetate buffer was used at a flow rate of 0.7 ml/min. The column effluent was monitored at 215 or 235 nm. The compounds were quantified by measuring the peak heights in relation to those of standards chromatographed under the same conditions.

The reactions of compounds 9–11 in aqueous sodium hydroxide solutions were initiated by adding 100 μl of a stock solution of the compounds in acetonitrile to 10 ml of preheated buffer solution, the final concentrations of the compounds being about 10^{-4} M. The solutions were kept in a water-bath at 37 °C and at appropriate intervals samples of 250 μl were withdrawn and added to 500 μl of 0.05 M acetate buffer of pH 4 to quench the hydrolysis. A 20 μl aliquot portion of the mixtures were chromatographed.

In case of the hydrolysis studies performed in human plasma diluted to 80% with 0.05 M phosphate buffer of pH 7.4, the reactions were initiated as described above. At appropriate intervals samples of 250 μl were withdrawn and added to 500 μl of methanol or, in some cases, 500 μl of a 2% solution of zinc sulphate in methanol–water (1 : 1 v/v) in order to deproteinize the plasma. After immediate mixing and centrifugation for 3 min at 10,000 rpm, 20 μl of the clear supernatant was analyzed by HPLC as described above.

Results and Discussion

Kinetics of alkaline hydrolysis

The kinetics of hydrolysis of the various esters 1–18 was studied in aqueous sodium hydroxide solutions at 37 °C. Under the experimental conditions used all reactions displayed strict first-order kinetics over several half-lives (cf. Fig. 1) and for all compounds except the glycolate esters 9–11

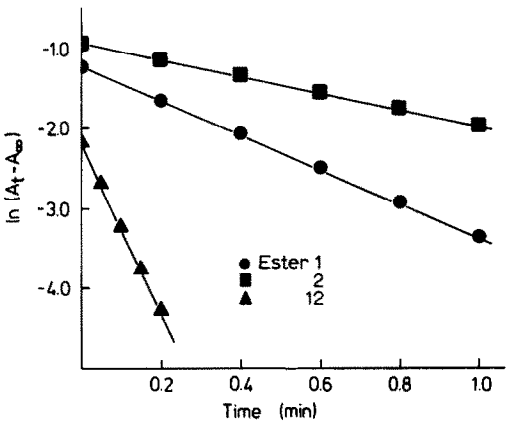


Fig. 1. First-order plots for the hydrolysis of the esters 1, 2 and 12 in 0.217 M sodium hydroxide at 37 °C as determined from direct spectrophotometric monitoring of the reaction progress.

benzoic acid was formed in stoichiometric amounts as revealed by HPLC analysis.

The rates of hydrolysis were found to be proportional to the hydroxide ion concentration or activity in the investigated range (0.001–0.22 M NaOH) in accordance with the following rate expression:

$$k_{obs} = k_{OH} a_{OH} \quad (1)$$

where k_{obs} is the observed pseudo-first-order rate constant and k_{OH} is the second-order rate constant for hydroxide ion-catalyzed hydrolysis. The hydroxide ion activity (a_{OH}) was calculated from the hydroxide ion concentration according to Harned and Hamer (1933). The values of k_{OH} for the various esters are listed in Table 2.

The hydrolysis of the *O*-benzoyl glycolate esters 9–11 was found to proceed by two parallel reactions as shown in Scheme 1. As revealed by HPLC analysis benzoylglycolic acid was formed in addition to benzoic acid and in amounts greatly exceeding those of benzoic acid. Fig. 2 shows a typical time-course for the various species in the degradation of ester 10. When the reactions of these esters were followed for a long period all the benzoylglycolic acid formed was found to hydrolyze to benzoic acid. In Scheme 1 k_1–k_4 refer to the pseudo-first-order rate constants for the

TABLE 2

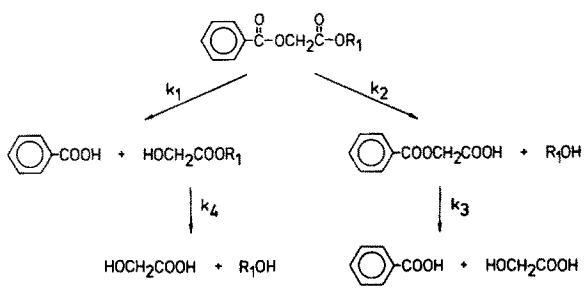
Rate data for the alkaline and enzymatic hydrolysis of various benzoic acid esters at 37 °C

Compound No.	—$COOCH_2$-R′ R′	σ * for R′	k_{OH} (M^{-1} min^{-1})	80% Human plasma k (min^{-1})	80% Human plasma $t_{1/2}$ (min)
1	H	0.49	13.6	6.4×10^{-3}	108
2	$-CH_3$	0.00	6.59	3.3×10^{-3}	210
3	$-C_2H_5$	−0.10	5.52	1.5×10^{-2}	46
4	$-C_3H_7$	−0.12	4.50	1.7×10^{-2}	40
5	$-C_4H_9$	−0.25	5.33	2.9×10^{-2}	24
6	$-C_6H_5$	0.75	13.0	3.7×10^{-2}	19
7	$-CH_2C_6H_5$	0.27	4.63	4.7×10^{-2}	15
8	$-COO^-$	−1.06	6.28	$< 10^{-4}$	> 100 h
9	$-COOCH_3$	2.00	70.1 [a]	2.0×10^{-1}	3.5
10	$-COOC_2H_5$	2.26	60.3 [a]	4.4×10^{-2}	16
11	$-COOCH_2C_6H_5$	–	55.7 [a]	2.7×10^{-1}	2.6
12	$-CONH_2$	1.68	69.9	1.7×10^{-2}	40
13	$-CON(CH_3)_2$	1.94	19.2	> 5.0	< 8 s
14	$-SCH_3$	1.56	24.4	3.1×10^{-2}	22
15	$-SOCH_3$	2.88	274	5.9×10^{-1}	1.2
16	$-SO_2CH_3$	3.68	592	5.8×10^{-1}	1.2
17	$-CH_2N(CH_3)_2$	0.49	9.83	> 8.0	< 5 s
18	$-CH_2N(CH_3)_3^+$	1.90	95.1	> 8.0	< 5 s
19	$-NHCOC_6H_5$	1.68	2×10^6	5.2	8 s [b]

[a] These rate data are for the degradation route yielding benzoic acid (the k_1-reaction in Scheme 1).
[b] In pH 7.4 buffer without plasma the half-life for the decomposition of ester 19 is 30 s.

processes indicated. Since k_3 is much smaller than the rate constant for the overall loss of ester ($k_{obs} = k_1 + k_2$) the rate constants k_1 and k_2 were simply determined on the basis of the relative amounts of benzoic acid and benzoylglycolic acid formed during the initial 2–3 half-lives of ester hydrolysis.

The exact expression for the time-course of benzoylglycolic acid (BG) arising during the hy-



Scheme 1

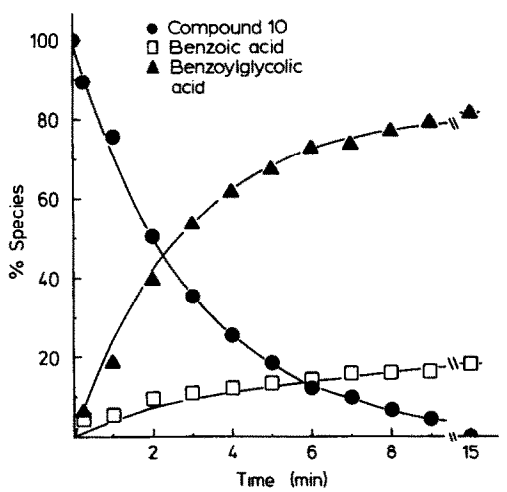


Fig. 2. Time courses for ethyl *O*-benzoylglycolate (compound 10), benzoic acid and benzoylglycolic acid during the degradation of compound 10 in 1.09×10^{-3} M sodium hydroxide solution ($\mu = 0.5$) at 37 °C.

drolysis according to Scheme 1 is:

$$\text{mol\% BG}_t = \frac{k_2 \cdot 100}{k_{obs} - k_3}(e^{-k_3 t} - e^{-k_{obs} t}) \qquad (2)$$

In Fig. 2 the full curve is constructed on the basis of this equation and the following values of the rate constants: $k_{obs} = 0.339$ min^{-1}; $k_2 = 0.292$ min^{-1}; $k_3 = 0.0049$ min^{-1} (values for 1.09×10^{-3} M NaOH solutions at 37 °C). From the identity: $k_{obs} = k_1 + k_2$, a value of 0.047 min^{-1} can be calculated for k_1.

The specific base catalytic rate constants associated with the $k_1 - k_3$ reactions are listed in Table 3.

As can be seen from the data the *O*-benzoylglycolate esters are most susceptible to undergo hydrolysis at the terminal acyl moiety, the k_2 rate constant being 4–16 times greater than the rate constant k_1 for the esters 9–11. These findings are in accordance with earlier results obtained in ethanol–water solutions (Ringshaw and Smith, 1964). These workers studied the products of reaction of various ethyl *O*-acylglycolates in 80% ethanolic potassium hydroxide solutions and found benzoylglycolic acid to be formed in 72% yield from ethyl *O*-benzoylglycolate (ester 10). The yield of the acid found in the present study using aqueous solutions is 86%.

Structural effects on alkaline hydrolysis rate

The rate of alkaline hydrolysis of esters is generally influenced by steric and polar effects within the acyl and alcohol moieties (Charton, 1977). The reactivity of the esters studied is primarily determined by the polar effects exhibited by the alcohol portions of the esters. The steric requirements in the alcohol portions of the esters can be considered to be almost constant (there is a methylene group connected to oxygen in all compounds) and with a few exceptions the variation of the rates of hydrolysis can be accounted for in terms of the different stability of the leaving alcohol group as expressed by the pK_a values of the alcohols. As seen in Fig. 3 a good linear correlation exists between log k_{OH} and the Taft polar substituent parameter σ *, the latter referring to R in RCH_2OH for the alcohols. Taft (1953) and others (Perrin et al., 1981) have previously reported that the pK_a of alcohols is linearly related to σ * used in this manner. The regression equation between log k_{OH} and σ * for the benzoate esters studied is given by:

$$\log k_{OH} = (0.54 \pm 0.04)\sigma^* + (0.74 \pm 0.07)$$

$$(n = 16;\ r = 0.962;\ F = 175) \qquad (3)$$

The k_{OH} values used for the glycolate esters 9–11 are the k'_{OH} values from Table 3 since these represent the hydrolysis of the benzoate ester moiety. This linear free-energy relationship may

TABLE 3

Specific base catalytic rate constants (in M^{-1} min^{-1}) for the hydrolysis of the glycolate esters 9–11 at 37 °C

Ester	k^1_{OH}	k^2_{OH}	k^3_{OH}	k_{OH} *
9	70.1	807	6.28	877
10	60.3	371	6.28	431
11	55.7	872	6.28	928

The k_{OH} constants refer to the processes depicted in Scheme 1.

* This k_{OH} value refers to the overall degradation of ester, i.e. $k_{OH} = k^1_{OH} + k^2_{OH}$.

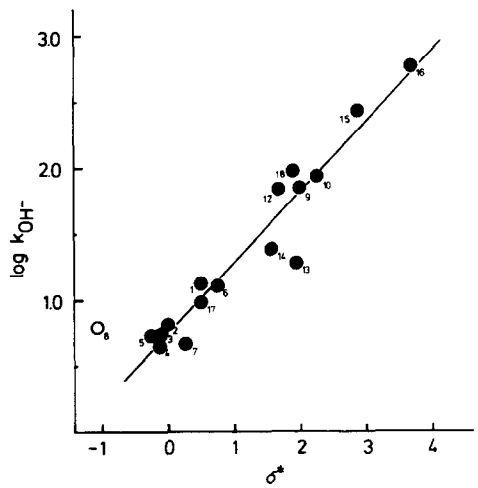


Fig. 3. Plot of log k_{OH} against the Taft polar substituent parameter σ * for various benzoate esters. Compound 8 was excluded from the regression line.

be useful for the prediction of the reactivity of a benzoate ester derivative solely on basis of the σ * value of the appropriate alcohol substituent. A large number of σ * values are available and have been compiled by Perrin et al. (1981). Robinson and Matheson (1969) have previously described a similar linear free-energy relationship between alcohol pK_a and hydrolysis rate of various esters. It is to be noted that the relationship described in Eqn. 3 covers esters with more than a 100-fold variation in reactivity.

The *N,N*-dimethylglycolamide ester 13 shows a somewhat lower reactivity than predicted from the correlation line in Fig. 3. Although steric effects by the two *N*-methyl groups may contribute to this some doubt may also be given to the σ * value reported for $-CON(CH_3)_2$ by Perrin et al. (1981). The value is not expected to be greater than that for $-CONH_2$ as it is actually reported in the compilation by Perrin et al. Boudreau and Williams (1977) have previously reported that intramolecular electrophilic assistance in the form of hydrogen bond donation to the ester from the amide NH in *O*-acylglycolamides does not operate during alkaline hydrolysis of such ester amides. The present finding that the reactivity of o-benzoylglycolamide (compound 12) can be totally accounted for in terms of polar effects lends further evidence to this conclusion.

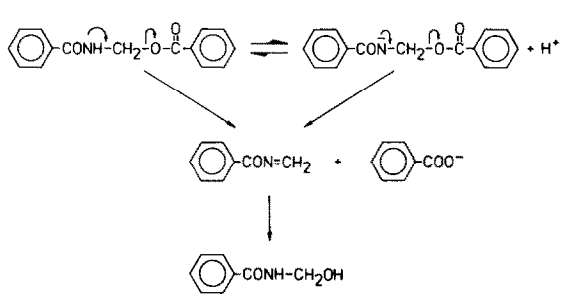
As seen from Fig. 3, benzoylglycolic acid (8) shows a positive deviation from the correlation plot. This is unexpected since a nucleophilic attack by hydroxide ions on the ester moiety should in fact be electrostatically hindered by the negatively charged carboxy group. The higher reactivity than predicted of ester 8 may possibly be due to some kind of intramolecular catalysis by the carboxy group. The hydrolysis of benzoylglycolic acid has previously been studied by Arcelli and Concilio (1977) and a rate enhancement occurring at pH 4–6 was ascribed to intramolecular general base-catalyzed hydrolysis by the ionized carboxylate group.

The k_{OH} value for the methylthiomethyl benzoate (ester 14) is close to the correlation plot in Fig. 3. This indicates that its hydroxide ion-catalyzed hydrolysis is a normal S_N2 reaction. The pH-rate profile for this ester exhibits a large plateau at pH 2.5–7 with the hydrolysis rate being pH-independent and at these pH values the compound most likely hydrolyzes via a unimolecular alkyl-oxygen cleavage (Loftsson and Bodor, 1981).

Considering N-(benzoyloxymethyl)benzamide (ester 19) its k_{OH} value (Table 2) is far larger than predicted on the basis of Eqn. 3. The σ * value for the substituent $-NHCOC_6H_5$ is 1.68 (Perrin et al., 1981) and from Eqn. 3 a k_{OH} value of 44.2 M^{-1} min^{-1} is calculated. This figure is seen to be much lower than the value (2×10^6 M^{-1} min^{-1}) actually determined. The large difference is strongly indicative of the involvement of another mechanism of hydrolysis for this ester, which also shows a very high pH-independent rate of decomposition at pH 2–6 ($t_{1/2}$ about 14 min). As has recently been described for related esters (Overton et al., 1986; Bundgaard and Buur, 1987) a mechanism involving an unimolecular elimination-addition process with the formation of a transient N-acylimine intermediate appears most likely (Scheme 2). The results of a more detailed study of the chemical reactivity of ester 8 and related compounds will be described in a future communication.

Enzymatic hydrolysis

The rates of hydrolysis of the esters 1–19 were determined in 80% human plasma (pH 7.4) and 37 °C. For all compounds except the esters 13, 17 and 18 good first-order kinetics were observed over several half-lives. Typical first-order plots are shown in Fig. 4. The rate of hydrolysis of the esters 13, 17 and 18 initially followed zero-order kinetics and as the ester substrate depleted it changed to follow first-order kinetics. A plot of



Scheme 2

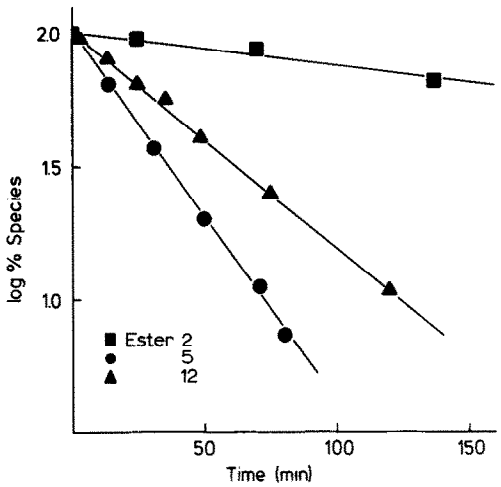


Fig. 4. First-order plots for the hydrolysis of the esters 2, 5 and 12 in 80% human plasma solutions at 37 °C.

the data from hydrolysis of ester 13 in 50% human plasma is shown in Fig. 5. As described elsewhere (Bundgaard and Nielsen, 1987) these progress curves can be described according to the integrated form of the Michaelis-Menten equation (Robinson and Characklis, 1984) from which the rate parameters K_m and V_{max} can be obtained. Analysis of the curve in Fig. 5 in this way resulted in a K_m value of 1.35×10^{-4} M^{-1} and a V_{max} value of 6.4×10^{-4} M^{-1} min^{-1}. The pseudo-first-order rate constant for the plasma-catalyzed hydrolysis equals V_{max}/K_m and the values obtained are listed in Table 2 along with the corresponding half-lives.

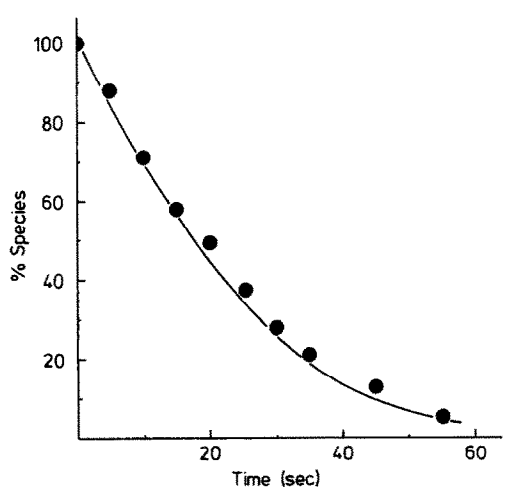


Fig. 5. Plot showing the zero-order followed by first-order rate of hydrolysis of 2-(benzoyloxy)-*N*,*N*-dimethylacetamide (ester 13) in 50% human plasma at 37 °C. The points refer to the percentage ester remaining at various reaction times.

Inspection of the data shows that the hydrolysis of the esters are strongly catalyzed by plasma enzymes although to varying extents. The half-lives of non-enzymatic hydrolysis at pH 7.4 and 37 °C can be estimated from the k_{OH} values. For example, the half-lives of hydrolysis of the esters 1, 13 and 16 are calculated to be 1.4×10^3, 2.0×10^3 and 32 h, respectively. As described previously (Bundgaard and Nielsen, 1987) the extremely rapid plasma-catalyzed hydrolysis of the *N*,*N*-dimethylglycolamide ester is due to cholinesterase (EC 3.1.1.8; also called pseudocholinesterase or butyrylcholinesterase) and the same enzyme is most likely also responsible for the rapid hydrolysis of benzoylcholine (ester 18) and its tertiary amine analog, ester 17.

For the design of prodrug esters the overall rate of hydrolysis in plasma or blood is of major interest. As noted in the Introduction simple alkyl esters may sometimes only be hydrolyzed rather slowly by plasma enzymes. This is thus the case for the methyl and ethyl esters of benzoic acid, the half-lives being about 2 and 3.5 h, respectively. By increasing chain length in the alkyl esters an increased enzymatic reactivity is seen except when going from methyl to ethyl. The ethyl ester is the least reactive ester and preliminary studies have indicated that this also holds true for the plasma-catalyzed hydrolysis of aliphatic esters of other carboxylic acids. This finding may be of relevance in prodrug design. Thus, if a rapid prodrug →

TABLE 4

Amount of benzoic acid formed upon degradation of the glycolate esters 9–11 in alkaline aqueous solution and human plasma at 37 °C

Compound	% Benzoic acid formed	
	Aqueous NaOH	80% Plasma (pH 7.4)
9	8.0	91.0
10	14.0	82.0
11	6.0	92.0

drug conversion in the blood is desired, a situation encountered with prodrugs intended to improve the absorption of drugs, an ethyl ester is not the optimal choice.

The methylsulfinylmethyl (15) and methylsulfonylmethyl (16) esters are readily hydrolyzed by plasma enzymes. Such esters have been proposed as prodrugs of acetylsalicylic acid (Loftsson et al., 1981) but a limitation with the esters may be their rather high chemical reactivity. The same applies to *O*-acylglycolate esters (compounds 9–11). These esters are further characterized by showing an incomplete conversion to the parent acid (benzoic acid) in plasma. Analogously to the hydroxide ion-catalyzed hydrolysis the glycolate esters were found to be cleaved concurrently at their two ester moieties in the plasma solutions according to Scheme 1. In contrast to the chemical hydrolysis, however, the k_1-reaction affording benzoic acid predominated over the k_2-reaction giving benzoylglycolic acid in the enzymatic hydrolysis. Fig. 6 shows the time-course for these acids during the hydrolysis of compound 10 in 80% plasma solutions and in Table 4 the amounts of benzoic acid produced from the esters in the enzyme- as well as the chemical-mediated hydrolysis are listed. Thus, although the glycolate esters show a rather high rate of enzymatic hydrolysis the conversion to the parent acid may not be quantitative. The product arising from hydrolysis at the terminal ester moiety, benzoylglycolic acid, is highly resistant to enzymatic hydrolysis, the half-life being > 100 h in human plasma. Glycolate esters have previously been suggested as prodrug forms of various carboxylic acid agents including amino acids and peptides (Wermuth, 1980) but no data have apparently been published about their enzymatic cleavage.

The high resistance of benzoylglycolic acid towards enzymatic hydrolysis by plasma may be due to its negative charge at physiological pH. Other esters with an ionized carboxylate group are also poor substrates for hydrolytic enzymes, e.g. succinate esters of metronidazole (Johansen and Larsen, 1984), various corticosteroids (Melby and Cyr, 1961; Anderson et al., 1985), chloramphenicol (Strebel et al., 1980; Ambrose, 1984; Kramer et al., 1984), *N*-hydroxymethyl-chlorzoxazone (Johansen and Bundgaard, 1981) and silybin (Koch and Tscherny, 1983), and as a consequence thereof, the bioavailability of the parent drug from these esters are often reduced. Similarly, acemetacin (compound 21; Scheme 3) which is a clinically used prodrug of indomethacin (20) formed by esterification with glycolic acid, is not readily cleaved by plasma enzymes and is only partly converted to indomethacin following oral administration (Dell et al., 1980).

It appears that glycolamide esters are much more suitable prodrug forms than glycolate esters in view of both enzymatic conversion, chemical stability and physical properties (Bundgaard and Nielsen, 1987). Thus, the present data show that the glycolate esters 9–11 are 25–50 times more unstable than the glycolamide ester 13, but at the same time are cleaved at a slower rate by plasma

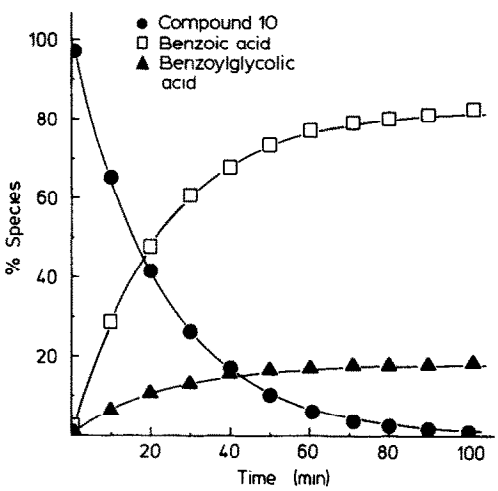
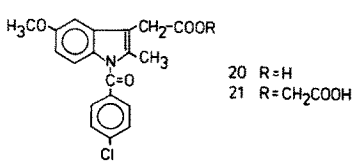


Fig. 6. Time courses for ethyl *O*-benzoylglycolate (compound 10), benzoic acid and benzoylglycolic acid during the degradation of compound 10 in 80% human plasma at 37 °C.



Scheme 3

enzymes. This greatly diminished stability of the glycolate esters stems primarily from facile hydrolysis of the terminal acyl-activated ester moiety (the k_2-reaction in Scheme 1). The results of a detailed study on the enzymatic and chemical hydrolysis of various glycolamide esters will be reported later.

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