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Hemiesters of aliphatic dicarboxylic acids as cyclization-activated prodrug forms for protecting phenols against first-pass metabolism

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Abstract

Various hemiesters of aliphatic dicarboxylic acids and paracetamol or phenol were prepared and assessed as potential prodrug forms with the aim of protecting phenolic drugs against first-pass metabolism after peroral administration. The degradation of the hemiesters was studied in aqueous buffer solutions and in various biological media. In buffer solutions the derivatives degraded via an intramolecular ring-closure reaction in addition to acid- or base-catalyzed ester hydrolysis. At physiological pH (7.4) the intramolecular ring-closure reaction is the major reaction contributing to the overall degradation and half-lives in the range of 1–350 min at pH 7.4 and 37° C were determined for the studied derivatives. The half-lives depended upon the degree of substitution in the acid moiety, the size of the formed anhydride and the pK_a value of the parent phenol. Liver homogenates clearly catalyzed the degradation of the hemiesters, whereas plasma showed different results for the esters of paracetamol and phenol, respectively. The only derivative not subjected to any enzymatic catalysis was the hemi-3,3-dimethylglutarate of paracetamol. Although highly substituted hemiesters might be useful prodrugs for certain individual phenolic drugs, derivatization of phenolic drugs into hemiesters may not be a generally useful prodrug approach for protection against first-pass metabolism, due to the fact that the derivatives are subjected to marked enzymatic catalysis in liver homogenates.

Keywords: Prodrug; First-pass metabolism; Phenol; Paracetamol; Cyclization; Glutarate; Succinate; Stability; Hemiester

1. Introduction

It is well known that extensive first-pass metabolism occurs to a large number of phenolic drugs like morphinans, steroids and salicylates, resulting in a low and variable bioavailability after peroral administration. First-pass metabolism of phenolic drugs occurs mainly in the gut mucosa and/or the liver, the major metabolic pathways being sulphation, methylation or glucuronidation of the phenolic moieties (George,

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1981; Mulder, 1982; Pond and Tozer, 1984; Ilett et al., 1990). The prodrug principle may be a useful approach to protect the vulnerable phenolic group against first-pass metabolism. A prerequisite for the usefulness of the prodrug approach is that demasking of the protective group occurs mainly in an organ other than the intestine or liver (Svensson and Tunek, 1988; Lokind et al., 1991; Bundgaard, 1992). If the demasking occurs by enzymatic reactions in these organs, the active parent drug may subsequently be metabolized within the same organ, and no protection of the phenolic group will be achieved. A more promising prodrug approach to prevent or reduce the first-pass metabolism of phenolic drugs may be the use of prodrug derivatives, in which the conversion to the parent phenolic drug occurs by non-enzymatic means, e.g., by chemical hydrolysis or an intramolecular reaction occurring with an appropriate rate at physiological pH (7.4) and 37° C. To ensure passage of the prodrug in largely intact form through the stomach and upper intestine, suitable prodrug forms should preferably be more stable at lower pH values. Studies to exploit this prodrug approach have been initiated in our laboratory (Hansen et al., 1992; Thomsen and Bundgaard, 1993; Thomsen et al., 1994) as well as by others (Saari et al., 1990; Patel et al., 1991; Getz et al., 1992). In the present work hemiesters of aliphatic dicarboxylic acids and paracetamol and phenol (used as model compounds for phenolic drugs) have been prepared and evaluated as possible prodrug forms (Table 1).

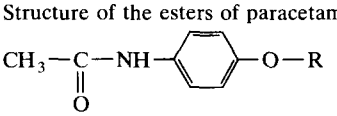
It has previously been shown that this type of hemiesters of aliphatic dicarboxylic acids and phenols degrades mainly via an intramolecular ring-closure reaction at physiological pH (Bruice and Pandit, 1960; Gaetjens and Morawetz, 1960). Esters containing a negatively charged group near the ester bond are often poor substrates for esterases (Krisch, 1971; Schöttler and Krisch, 1974; Nielsen and Bundgaard, 1987 and references cited therein). Also, the rate of enzymatic hydrolysis of phenyl esters is reduced if the acyl chain is branched (Digenis and Swintosky, 1975; Wagner et al., 1980). It was thought that the ionized carboxylate moiety at pH 7.4 together with substituents in the acid moiety of the hemiester could hinder enzymatic hydrolysis in biological media, so that the intramolecular ring-closure reaction would alone be responsible for the degradation of the compounds. The present work was carried out to investigate further the usefulness of hemiesters of aliphatic dicarboxylic acids as prodrugs of phenolic drugs.

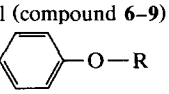
2. Materials and methods

2.1. 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 Rheodyne 7125 injection valve with a 20 μl loop. A deacti-

Table 1
Structure of the esters of paracetamol (compound **1–5**) and phenol (compound **6–9**)





Compound	R	m.p. (° C)	Compound	R	m.p. (° C)
1	$COCH_2CH_2CH_2COOH$	174–176	**6**	$COCH_2CH_2CH_2COOH$	44–45 [b]
2	$COCH_2CH(CH_3)CH_2COOH$	137–138	**7**	$COCH_2CH(CH_3)CH_2COOH$	50–53
3	$COCH_2C(CH_3)_2CH_2COOH$	146–147	**8**	$COCH_2OCH_2COOH$, H_2O	43–46
4	$COCH_2OCH_2COOH$	172–174	**9**	$COCH_2CH_2COOH$	88–91 [c]
5	$COCH_2CH_2COOH$, H_2O	143–146 [a]			

[a] Reported m.p. 136–137° C (Gaetjens and Morawetz, 1960); reported m.p. 145.5–146.5°C (Dittert et al., 1968).
[b] Reported m.p. 45–46° C (Gaetjens and Morawetz, 1960).
[c] Reported m.p. 98° C (Gaetjens and Morawetz, 1960).

vated Supelcosil LC-8-DB reversed-phase column (33 × 4.6 mm i.d.) (3 μm particles) from Supelco Inc., USA, was used in conjunction with a Supelguard precolumn. In some cases, a Kontron system consisting of an LC pump T-414, a Uvikon 740 UV detector operated at a fixed wavelength (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. Readings of pH were carried out on a Radiometer PHM Autocal instrument at the temperature of the study. Elemental analysis was performed at Leo Pharmaceuticals, Ballerup, Denmark.

2.2. Chemicals

Phenol, glutaric anhydride, 3-methylglutaric anhydride, 3,3-dimethylglutaric anhydride, diglycolic anhydride and succinic anhydride were all purchased from Aldrich Chemie, Germany. Paracetamol was obtained from Mecobenzon A/S, Copenhagen. Buffer substances and solvents used were of reagent grade.

2.3. Preparation of hemiester derivatives

The hemiesters of various dicarboxylic acids and paracetamol and phenol (Table 1) were prepared according to the following general procedure. Paracetamol/phenol (10 mmol) was dissolved in 20 ml of ice-cold 0.5 M sodium hydroxide. This was followed by the addition of 10 mmol of the appropriate anhydride. The reaction mixture was stirred for 10–15 min in an ice-bath and then acidified with 4 M hydrochloric acid. The resulting precipitate (compounds **1–5**, **9**) was filtered, dried and recrystallized several times from ethanol-water (compounds **2–4**, **9**) or ethanol-water-acetone (compound **1**) or ethanol-*n*-pentane (compound **5**). Compound **5** was obtained as a monohydrate. For compounds **6** and **7** acidification of the reaction mixture resulted in the formation of an oily phase, which was taken up in ethyl acetate. The solution was dried over anhydrous sodium sulphate, filtered and evaporated in vacuo. The residue obtained was recrystallized from methylene chloride-petroleum ether. For compound **8** precipitation was achieved before acidification of the reaction mixture. The precipitate isolated was recrystallized twice from ethanol-water, and the precipitate was obtained as a mixture of the free acid and the sodium salt. The precipitate was dissolved in water and acidified, and the free acid was extracted in ethyl acetate. The ethyl acetate extract was dried with sodium sulphate, filtered and evaporated in vacuo. The residue obtained was recrystallized from ethyl acetate-petroleum ether and compound **8** was obtained as a monohydrate. Melting points (uncorrected) for the derivatives are listed in Table 1. For all compounds the elemental analyses (C,H and N) were within ±0.4% of the calculated values.

2.4. Calculations

For the sake of simplicity the *p*-acetamidophenyl group in compounds **1**, **3** and **4** was replaced with a methyl group in the energy calculations. The energies of the stretched and the folded conformation were found after optimization of the structure by a molecular mechanics calculation (Version 3.6) using the Cache program package. Prior to optimization of the folded conformations their carboxylate oxygen atom and ester carbonyl carbon atom were fixed orthogonally at a distance of 2 Å. The energy difference between the folded and the stretched conformer was calculated to be 20.068–6.237 = 13.831 kcal/mol for compound **3**, 30.712–15.962 = 14.751 kcal/mol for compound **4** and 16.711–1.097 = 15.614 kcal/mol for compound **1**.

2.5. Kinetic measurements

2.5.1. Degradation in aqueous solutions

The degradation of compounds **1–9** was studied in aqueous buffer solutions at constant temperature (±0.2° C). The buffers used were hydrochloric acid (pH 1–2), acetate (pH 4–5), phosphate (pH 3, 6–7.4), borate (pH 8.5–10) and carbonate (pH 11) solutions at a total buffer concentration of 0.02 M. A constant ionic strength

(μ) of 0.5 was maintained for each buffer solution by adding the calculated amount of potassium chloride.

The rates of degradation of the compounds were determined by using reversed-phase HPLC procedures capable of separating the compounds from their degradation products. Mobile phase systems of 5–25% (v/v) acetonitrile in 0.1% (v/v) phosphoric acid were used with triethylamine added at a concentration of 10^{-3} M to improve peak shape. The concentration of acetonitrile was adjusted for each compound to give a retention time of 2–10 min. The column effluent was monitored at 215 nm.

The degradation reactions in buffer solutions were initiated by adding 100 μl of a stock solution of the compounds in acetonitrile to 10 ml of preheated buffer solution in screw-capped test tubes, the final concentration being $1-2 \times 10^{-4}$ M. The solutions were kept in a water-bath at constant temperature 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 compound against time.

2.5.2. Degradation in biological media

The degradation of the compounds was studied at 37° C in 40% human plasma, 20% pig liver homogenate and 20% rat liver homogenate. The liver homogenates were prepared by homogenizing the liver tissue in ice-cold 0.05 M phosphate buffer. The homogenates (50%) were frozen in samples of 2 ml until use, where the final homogenates (20%) were prepared by dilution with 0.05 M phosphate buffer. The initial concentration of the compounds were about $1-2 \times 10^{-4}$ M. The reaction mixtures 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 a 2% (w/v) solution of zinc sulphate in methanol-water (1:1 v/v) in order to stop the reactions and deproteinize the sample. After mixing and centrifugation for 3 min at 13000 rpm, 20 μl of the clear supernatant was analyzed by HPLC as described above.

3. Results and discussion

3.1. Kinetics of degradation

All the hemiester derivatives studied underwent quantitative degradation to paracetamol (**1–5**) or phenol (**6–9**) in aqueous buffer solutions. The degradation reactions displayed strict first-order kinetics at constant pH and temperature. The influence of pH on the overall rates of degradation of compounds **3** and **7** is shown in Fig. 1. The different shape of the curves for compounds **3** and **7** is due to both variation in the phenol residue and the substituent pattern in the acid moiety.

The pH-rate profile for compound **3** can be described by an apparent acid-catalyzed hydrolysis of the unionized hemiester and a spontaneous degradation of the ionized hemiester as reflected by the following rate expression:

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

where k_{obs} is the observed first-order rate constant, a_H denotes the hydrogen ion activity, k_H is the second-order rate constant for the apparent

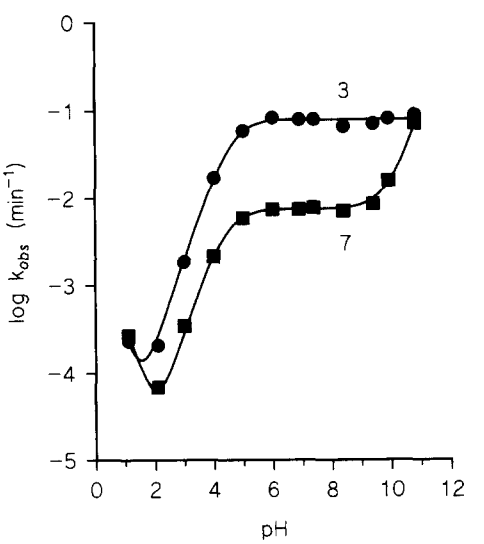


Fig. 1. The pH-rate profiles for the degradation of compounds **3** (●) and **7** (■) in aqueous buffer solutions at 37° C.

specific acid-catalyzed hydrolysis, and k_0 represents the first-order rate constant for the spontaneous degradation of the ionized hemiester. $a_H/(a_H + K_a)$ and $K_a/(a_H + K_a)$ are the fractions of total hemiester on the acid and the ionized form, respectively, and K_a denotes the apparent ionization constant. For compound **7** an additional term corresponding to a base-catalyzed hydrolysis of the ionized hemiester is needed to account for the observed pH-rate profile. The pH-rate profile for compound **7** can be accounted for by the following rate expression:

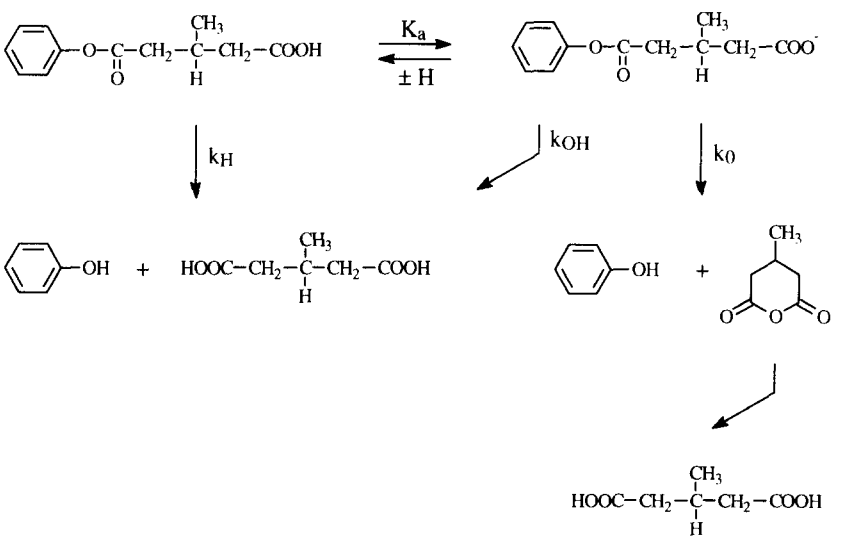
$$k_{obs} = k_H a_H \frac{a_H}{a_H + K_a} + (k_0 + k_{OH} a_{OH}) \frac{K_a}{a_H + K_a} \quad (2)$$

where a_{OH} is the hydroxide ion activity and k_{OH} represents the second-order rate constant for the apparent specific base-catalyzed hydrolysis. The values obtained for these rate constants at 37°C were: compound **3**, $k_H = 0.0025\ M^{-1}\ min^{-1}$, $k_0 = 0.08\ min^{-1}$, $pK_a = 4.6$; and for compound **7**, $k_H = 0.0035\ M^{-1}\ min^{-1}$, $k_0 = 0.0075\ min^{-1}$, $k_{OH} = 40\ M^{-1}\ min^{-1}$, and $pK_a = 4.4$.

3.2. Mechanism of degradation

Phenyl esters of carboxylic acids exhibit in general good chemical stability, having typical U-shaped pH-rate profiles corresponding to specific acid- or base-catalyzed hydrolysis. An intramolecular nucleophilic attack of the ionized carboxyl group on the ester function is responsible for the rapid degradation displayed by the hemiesters studied. Their degradation can therefore be described by the usual acid- or base-catalyzed hydrolysis together with an intramolecular ring-closure reaction (as exemplified for compound **7** in Scheme 1). The intramolecular nucleophilic attack results in the formation of the parent phenol and an anhydride, which is subsequently degraded to a dicarboxylic acid (Bruice and Pandit, 1960). In this study only the degradation of the hemiesters has been studied and no attempts were made to follow the formation and degradation of the anhydrides.

The use of hemiesters of dicarboxylic acids and paracetamol in the context of prodrugs has previously been studied (Dittert et al., 1968; Rattie et al., 1970; Kovach et al., 1981). In these studies the primary purpose with the formation of



Scheme 1.

the prodrugs was to improve the physico-chemical properties of paracetamol, e.g., its stability or solubility.

3.3. Factors influencing rate of degradation

Table 2 lists the observed half-lives of degradation of the compounds studied at pH 7.4 and 37° C. At pH 7.4 the intramolecular ring-closure reaction is the major reaction contributing to the overall degradation. The half-lives at pH 7.4 can therefore be compared to obtain information about the factors, which influence the rate of the intramolecular ring-closure reaction for this kind of compounds.

The reactivity of these hemiesters is determined by three factors, namely, the size of the formed intramolecular anhydride, the number of substituents in the acid moiety and the pK_a value of the phenolic group. The succinate esters react 133- and 151-times faster than the glutarate esters (cf. compounds **1/5** and **6/9**). These factors correspond well with the results obtained by others (Bruice and Pandit, 1960; Gaetjens and Morawetz, 1960). The greater reactivity of the succinates can be explained by the proximity of the reacting groups. Substituents in the acid moiety result in a greater reactivity. Changing from no substituents to one methyl group (compound **1** → **2**, **6** → **7**) increases reactivity by a factor of 3.5–3.8, whereas changing to two methyl groups (compund **1** → **3**) increases reactivity 18-times. These results agree nicely with those reported by Bruice and Pandit (1960), who found a 4.41- and 19.3-fold increase in reactivity of *p*-bromophenyl 3-methylglutarate and *p*-bromophenyl 3,3-dimethylglutarate as compared to *p*-bromophenyl glutarate. They explained the greater reactivity of the substituted compounds on the basis of an increase of rotamers in a favorable position for the intramolecular reaction. The difference of the rate constants obtained for compounds **1** and **3** corresponds to a difference of approx. 1.78 kcal/mol at 37° C in the activation energy. This energy difference agrees well with the difference for compounds **1** and **3** in the calculated conformational energy difference between the stretched conformer and the folded conformer required in the transition state. This means that the energy required for going from a stretched state to the folded transition state is smaller for compound **3** than for compound **1**, e.g., the substituents force the two reacting groups together resulting in an increase in the cyclization rate. The substituent in the phenol group also influences the rate of degradation. The esters of paracetamol are degraded 1.6–2.4-times more rapidly than the corresponding esters of phenol (cf. compounds **1/6**, **2/7**, **4/8** and **5/9**). This corresponds well with the result from the study by Gaetjens and Morawetz (1960), which led to the conclusion that the rate of the intramolecular reaction depended upon the leavability of the phenoxide ion. The rate-limiting step in the cyclization reaction involves extrusion of the phenoxide ion, and this explains the dependence on leavability. The pK_a value for paracetamol is 9.49, whereas it is 9.86 for phenol (Kovach et al., 1981). Therefore, the

Table 2
Half-lives of degradation of compounds **1–9** in aqueous buffer solutions (pH 7.4) and biological media at 37° C

Compound	$t_{1/2}$ (min)			
	pH 7.4 buffer	40% human plasma	20% pig liver homogenate	20% rat liver homogenate
1	160	148	0.6	0.1
2	42	41	1.7	3.0
3	8.9	9.7	7.1	8.3
4	69	55	0.6	0.3
5	1.2	0.9	0.5	0.4
6	348	121	0.1	0.2
7	100	204	0.6	0.6
8	111	28	0.2	0.2
9	2.3	1.0	0.2	0.4

anion of paracetamol is a better leaving group than the phenoxide ion, and this explains the greater reactivity of the esters of paracetamol. That the susceptibility of hemiesters to undergo intramolecular ring-closure reaction catalyzed by the terminal carboxylate group is dependent on the basicity of the hydroxy group has been described previously (Johansen and Larsen, 1984). Hemiesters of dicarboxylic acids and metronidazole (pK_a about 15.9) degraded without intramolecular reaction, whereas 21-hemiesters of dicarboxylic acids and hydrocortisone (pK_a values about 11, due to enolization) were subjected to an intramolecular ring-closure reaction (Johansen and Larsen, 1984 and references cited therein). Finally, compounds **4** and **8**, in which a methylene group has been substituted with an oxygen atom, are more reactive than compounds **1** and **6**. The difference of the rate constants obtained for compounds **1** and **4** corresponds to a difference of approx. 0.52 kcal/mol at 37° C in the activation energy. The difference for compounds **1** and **4** in the calculated conformational energy difference between the stretched and the folded conformer is 0.863 kcal/mol. This means that if steric factors alone were responsible for the different reactivity of the two compounds, a greater difference in the obtained half-lives would be expected. The reason that this is not so might be the different electronic effects for the two type of compounds displayed by the oxygen atom.

3.4. Stability in biological media

The stability of the hemiesters was also studied in various biological media (pH 7.4, 37° C). The degradation process was found to conform closely with first-order kinetics, and paracetamol or phenol was released in quantitative amounts. The half-lives obtained for the degradation of the compounds in biological media are shown in Table 2. There was no sign of a catalytic influence of human plasma on the degradation of the esters of paracetamol (compounds **1–5**), whereas different results were obtained for the esters of phenol. For compounds **6**, **8** and **9** a catalytic effect, amounting to a factor of 2–4 in 40% human plasma was observed, while the degradation of compound **7** is 2-fold slower in human plasma than in buffer solution. This may be explained by plasma protein binding of the compound. Lack of in vitro plasma catalysis has been described for other hemiesters (Johansen and Larsen, 1984; Anderson et al., 1985), however, conflicting results concerning plasma catalysis have also been obtained (Larsen et al., 1988). The liver homogenates had a more pronounced effect on the degradation of the hemiesters. The compounds were subjected to significant enzymatic catalysis. The only exception was compound **3**. For compound **3** no enzymatic catalysis was seen in the liver homogenates and the half-lives obtained in buffer, plasma and liver homogenates were the same. From these results it may be predicted that the degradation of most of the compounds in human plasma proceeds via the intramolecular ring-closure reaction, and by enzyme-catalyzed hydrolysis in the liver homogenates. An important exception is compound **3**, which did not undergo enzymatic hydrolysis in human plasma or liver homogenates. The reason for this is most likely the combination of negative charge of the compound at physiological pH and the considerable steric hindrance in the acid moiety of this compound.

4. Conclusions

The results obtained in this study show that derivatization of the phenolic group in the form of hemiester of dicarboxylic acids does not appear to be a generally useful approach for protecting phenolic drugs against first-pass metabolism. The derivatives studied degraded via an intramolecular ring-closure reaction with appropriate half-lives at pH 7.4, but the derivatives underwent significant enzymatic catalysis in rat and pig liver homogenates. Thus, it appears that the negatively charged carboxylate group was not able to prevent enzymatic hydrolysis of the compounds in liver homogenates but partially in human plasma. Substituents in the acid moiety might to some extent prevent enzymatic catalysis as shown for the hemi-3,3-dimethylglutarate of paracetamol. This compound degraded with the same rate in buffer solution and biological media.

However, it may be anticipated that hemiesters of other phenolic drugs might have different resistance towards enzymatic catalysis.

Acknowledgements

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