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Aspirin prodrugs: synthesis and hydrolysis of 2-acetoxybenzoate esters of various *N*-(hydroxyalkyl)amides

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Summary

Three new esters of aspirin were synthesized and evaluated in vitro as potential prodrug forms of aspirin with the aim of depressing the gastrotoxicity of the drug by temporarily masking the carboxylic acid function. The esters, derived from *N*-(hydroxymethyl)acetamide, *N*-(hydroxymethyl)benzamide and α-hydroxy-*N*-benzoylglycine benzyl ester, were all found to undergo a facile hydrolysis in aqueous solution of pH 0–8 with a quantitative regeneration of aspirin. The compounds were very easily cleaved at pH 7.4 but were more stable at lower pH values. Due to the great lability at pH 7.4 the compounds were also found to be cleaved quantitatively or predominantly to aspirin in the presence of human plasma rather than to the corresponding salicylate esters and hence salicylic acid.

Introduction

For many years several attempts have been made to develop bioreversible derivatives or prodrugs of aspirin (acetylsalicylic acid) in order to depress gastric irritation and bleedings (Jones, 1985). On the premise that the gastric irritation and ulcerogenicity associated with oral dosing of aspirin is largely a local phenomenon (Ivey et al., 1980), a promising approach to minimize this side effect is masking the acidic carboxyl group of aspirin via prodrug formation. Upon administration, a successful prodrug derivative should pass intact through the stomach and first be hydrolyzed to aspirin in the intestine or, perhaps even better, during or following absorption.

The aspirin prodrug derivatives developed so far can be classified in two groups according to their mechanisms of conversion: derivatives which undergo enzymatic cleavage to regenerate the parent drug and derivatives being hydrolyzed non-enzymatically. The former group consists of several ester derivatives including simple alkyl or aryl esters (Rainsford and Whitehouse, 1980; Rainsford et al., 1980; Whitehouse and Rainsford, 1980; Cousse et al., 1978), triglycerides (Kumar and Billimoria, 1978; Paris et al., 1979, 1980), acyloxyalkyl esters (Los et al., 1982), certain sulphur-containing ester types (Loftsson et al., 1981; Loftsson and Bodor, 1981) and amides of phenylalanine derivatives (Banerjee and Amidon,

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1981a, b, c; Muhi-Eldeen et al., 1985). The non-enzymatically hydrolyzable derivatives include some acylal derivatives (Hussain et al., 1974, 1979; Truelove et al., 1980) and 2-substituted 2-methyl-4H-1,3-benzodioxin-4-one derivatives (Hansen and Senning, 1983). Other examples have been summarized by Jones (1985) and Loftsson et al. (1981).

A major problem in the design of aspirin prodrugs is, however, the great enzymatic lability of the acetyl ester functionality in aspirin derivatized at its carboxyl group. As recently shown in this laboratory (Nielsen and Bundgaard, in preparation) blocking of the carboxylic group (i.e. neutralization of the negative charge of the aspirin molecule) by e.g. esterification renders the acetyl group extremely susceptible to enzymatic cleavage. Thus, half-lives for the deacetylation of 1–3 min in human plasma were typically found for various aspirin esters. Therefore, a prerequisite for any true aspirin prodrug is that the masking group cleaves faster than the acetyl ester moiety as illustrated in Fig. 1. Otherwise, the derivatives will behave as prodrugs of salicylic acid and not as true aspirin prodrugs. With the apparent exception of methylsulfinylmethyl and methylsulfonylmethyl esters of aspirin (Loftsson et al., 1981) most or all of the considerable number of "aspirin prodrugs" which have been described in the literature do not fulfill this requirement of hydrolysis and they may essentially be regarded as prodrugs of salicylic acid rather than of aspirin (Muhi-Eldeen et al., 1985; Jones, 1985).

We recently discovered that various *N*-acyloxyalkyl derivatives of primary amides are

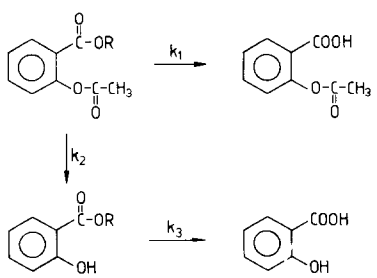
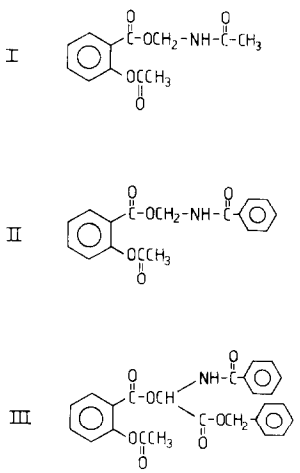


Fig. 1. Scheme illustrating the bioconversion of aspirin esters. To behave as true aspirin prodrugs the hydrolytic rate constant k_1 should be greater than the rate constant k_2 associated with deacetylation.



highly unstable in neutral aqueous solution (Bundgaard and Buur, 1987; Bundgaard and Nielsen, 1987). This finding led us to investigate such an ester type as being a potentially useful prodrug form for aspirin. To this end, esters of aspirin (Formulae I–III) derived from *N*-(hydroxymethyl)acetamide, *N*-(hydroxymethyl)benzamide and α-hydroxy-*N*-benzoylglycine benzyl ester have been prepared and their kinetics of hydrolysis investigated in aqueous solution and in human plasma.

Materials and Methods

Apparatus

Ultraviolet spectral measurements were performed with a Shimadzu UV-190 spectrophotometer equipped with a thermostated cell compartment, using 1-cm quartz cuvettes. Readings of pH were carried out on a Radiometer Type PHM 26 meter at the temperature of study. Melting points were taken on a capillary melting-point apparatus and are corrected. High-performance liquid chromatography (HPLC) was generally done with a Kontron apparatus consisting of an LC Pump T-414, a Uvikon 740 LC UV detector operated at a fixed wavelength (215 nm), a Rheodyne 7125 injection valve with a 20 μl loop and a Chrompack column (100 × 3 mm) packed with Chromspher C8 (5 μm particles). In some cases a Waters pump model 6000 A and a variable wavelength UV-detector (Waters Type Lambda Max 480) were used.

Microanalyses were performed by the Microanalytical Laboratory, Leo Pharmaceutical Products, Ballerup, Denmark.

Preparation of the derivatives

The following starting materials were prepared as described previously: α-hydroxy-*N*-benzoylglycine benzyl ester (Bundgaard and Buur, 1987); *N*-(hydroxymethyl)acetamide (Milkowski et al., 1980), m.p. 51–52 °C (from acetone), rep. m.p. 50–52 °C (Scharf, 1976); *N*-chloromethylbenzamide (Böhme et al., 1959). *O*-Acetylsalicyloyl chloride and acetylsalicylic acid were purchased from Fluka AG, Switzerland.

N-(O-Acetylsalicyloyloxymethyl)acetamide (I). A solution of *O*-acetylsalicyloyl chloride (2.38 g, 0.012 mol) in acetone (10 ml) was added dropwise over 15 min to a stirred solution of *N*-(hydroxymethyl)acetamide (0.89 g, 0.01 mol) in pyridine (10 ml) at 0–4 °C. The reaction mixture was stirred for 3 h at 0–4 °C and then kept overnight at 4 °C. Water (40 ml) and ethyl acetate (50 ml) were added and the organic phase separated, washed with 2 M hydrochloric acid, 5% sodium hydrogen carbonate and water. The ethyl acetate solution was dried over anhydrous sodium sulphate and evaporated under reduced pressure to leave a residue which crystallized from acetone–ether–petroleum ether at −20 °C to give 850 mg of compound I, m.p. 81–82 °C.

Anal.: Calc. for $C_{12}H_{13}NO_5$: C, 57.37; H, 5.22; N, 5.78. Found: C, 57.28; H, 5.25; N, 5.67.

N-(O-Acetylsalicyloyloxymethyl)benzamide (II). A mixture of acetylsalicylic acid (1.80 g, 0.01 mol), *N*-chloromethylbenzamide (1.70 g, 0.01 mol), triethylamine (1.4 ml, 0.01 mol) and sodium iodide (0.15 g, 0.001 mol) in ethyl acetate (50 ml) was refluxed with stirring for 4 h. Upon cooling, the mixture was filtered and the filtrate washed with 2 M hydrochloric acid, 5% sodium hydrogen carbonate and water. The ethyl acetate solution was dried over anhydrous sodium sulphate and evaporated under reduced pressure to give compound II as a solid. It was recrystallized from ethyl acetate–petroleum ether, yielding 2.2 g of white crystals, m.p. 128–129 °C.

Anal.: Calc. for $C_{17}H_{15}NO_5$: C, 65.17; H, 4.83; N, 4.47. Found: C, 65.12; H, 4.85; N, 4.50.

α-(O-Acetylsalicyloyloxy-N-benzoyl)glycine benzyl ester (III). A solution of *O*-acetylsalicyloyl chloride (0.60 g, 3 mmol) in acetone (4 ml) was added dropwise over 15 min to a stirred solution of α-hydroxy-*N*-benzoylglycine benzyl ester (0.855 g, 3 mmol) in pyridine (5 ml) at 0–4 °C. The reaction mixture was stirred for 3 h at this temperature and then kept overnight at 4 °C. The mixture was concentrated in vacuo and the residue was dissolved in ethyl acetate and washed with 2 M hydrochloric acid, 5% sodium hydrogen carbonate and water. The ethyl acetate solution was dried and evaporated in vacuo to leave a residue which crystallized by trituration with ether and storing at −20 °C for 2 days. The title compound was recrystallized from ethanol-ether-petroleum ether, yielding 0.65 g, m.p. 86–87 °C.

Anal.: Calc. for $C_{25}H_{23}NO_7$: C, 66.81; H, 5.16; N, 3.12. Found: C, 66.62; H, 5.23; N, 3.07.

Kinetic measurements

All rate studies were performed in aqueous buffer solutions at 37.0 ± 0.2 °C. The buffers used were hydrochloric acid, acetate, phosphate and borate buffers. A constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride.

The progress of the reactions was followed by direct UV-spectrophotometry or by HPLC. In the former method the reactions were performed in 2.5 ml aliquot portions of buffer solutions in a thermostated quartz cuvette and were initiated by adding 20 µl of stock solutions of the derivatives in acetonitrile to give a final concentration of $0.5–2 \times 10^{-4}$ M. The rate of hydrolysis of the compounds was followed by monitoring the decrease in absorbance at 240 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.

Except for the very rapid reactions the rates of degradation were in all cases followed by using reversed-phase HPLC procedures. Mobile phase systems of methanol in 0.01 M acetate buffer of pH 5.0 were used to determine the compounds I–III, the concentration of methanol being adjusted for each compound to give an appropriate

retention time (2–4 min). Thus, for compound III 70% v/v methanol was used. It was confirmed that the products of degradation had retention times different from those of the acetylsalicylic acid esters. The flow rate was 0.6–1.0 ml/min and the column effluent was monitored at 215 or 230 nm. Quantitation of the compounds was done by measuring the peak heights in relation to those of standards chromatographed under the same conditions. The reactions were initiated by adding 100 μl of a stock solution of the compounds in acetonitrile to 10 ml of preheated buffer solution in screw-capped test tubes, the final concentration of the compounds being about 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 derivative against time.

Hydrolysis studies were also performed in human plasma diluted to 80% with 0.05 M phosphate buffer of pH 7.40. In this case, samples of 250 μl were withdrawn and added to 1000 μl of ethanol in order to deproteinize the plasma. After immediate mixing and centrifugation for 2 min, 20 μl of the clear supernatant was analyzed by HPLC.

Analysis of the reaction solutions for acetylsalicylic acid formed was done with a mobile phase system consisting of 85% phosphoric acid–acetonitrile–methanol–water (3 : 10 : 20 : 67 v/v), the flow rate being 1.4 ml/min and the detection wavelength 230 nm. In this system acetylsalicylic acid showed a retention time of 2.3 min whereas that of salicylic acid was 4.2 min.

Results and Discussion

The kinetics of decomposition of the aspirin esters I–III was studied in aqueous solution at 37 °C over a wide range of pH. At constant temperature and pH the disappearance of the compounds displayed strict first-order kinetics over several half-lives (cf. Fig. 2). The rates of decomposition were found to be independent of buffer concentration at the concentrations used (0.01–0.03 M). The pseudo-first-order rate constants observed (k_{obs}) at various pH values are listed in Table 1. The pH-rate profiles for the derivatives are shown in Fig. 3. They can be accounted for by the following rate expression:

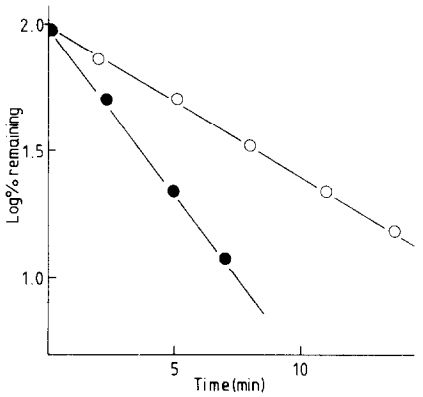


Fig. 2. First-order kinetic plots for the degradation of ester I in aqueous buffer solution of pH 5.00 (○) and of ester II at pH 6.02 (●).

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

where a_H and a_{OH} refer to the hydrogen ion and hydroxide ion activity, respectively. The latter was calculated from the measured pH at 37 °C according to the following equation (Harned and Hamer, 1933):

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

Values of the second-order rate constants for the

TABLE 1

Pseudo-first-order rate constants for the hydrolysis of the aspirin esters I–III in aqueous solution at 37 °C and μ = 0.5

pH	k_{obs} (min^{-1})		
	I	II	III
0 15	1.58	1.45	0.019
1.16	0.301	0.240	0.0035
2.00	0.141	0.125	0.0045
3.01	0.128	0.112	0.0095
4.00	0.139	0.120	0.060
5.00	0.140	0.152	0.63
6.02	0.159	0.295	6.4
6.90	0.279	1.41	
7.40	0.471	5.50	
8.20	4.95		

TABLE 2

Rate data for the hydrolysis of various acetylsalicylic acid esters in aqueous solution at 37 °C and $\mu = 0.5$

Compound	k_H (M^{-1} min^{-1})	k_0 (min^{-1})	k_{OH} (M^{-1} min^{-1})
I	2.40	0.14	1.05×10^6
II	2.00	0.12	8.91×10^6
III	0.016	0.0030	2.63×10^8

apparent specific acid (k_H) and specific base (k_{OH}) catalyzed decomposition were determined from the rate data at low and high pH values, respectively, whereas values of the apparent first-order rate constant for spontaneous decomposition (k_0) was obtained from the plateau regions of the pH–rate profiles. The value of the rate constants are listed in Table 2. In Fig. 3 the solid curves were constructed from these values and Eqn. 1.

The decomposition of the ester derivatives I–III proceeded at all pH values studied with the quantitative formation of aspirin as evidenced by HPLC analysis of the reaction solutions (Table 3). An example of a time course for the formation of aspirin in the degradation of ester I is shown in Fig. 4. As can be seen the formation of aspirin occurs according to first-order kinetics at constant pH. That the aspirin esters were exclusively hydrolyzed along the k_1-term depicted in Fig. 1 was further supported by UV-spectrophotometric

TABLE 3

Amount of aspirin formed upon decomposition of the aspirin esters I–III in aqueous buffer solutions and in human plasma solutions (pH 7.4) at 37 °C

Reaction solution	% Aspirin formed		
	I	II	III
pH 0.15	99	101	101
pH 2.00	100	99	99
pH 4.00	102	102	98
pH 7.40	101	100	100
80% plasma (pH 7.40)	80	98	100

scanning of the reaction solutions. No absorption peak or increase of absorbance was observed to occur at 295–305 nm, indicating the absence of any salicylate ester or salicylic acid formation. At longer reaction times, as expected, the HPLC measurements revealed the formation of small amounts of salicylic acid which is due to hydrolysis of the aspirin formed in the primary decomposition reaction.

Besides aspirin the corresponding *N*-(hydroxyalkyl)amides were found to be primary reaction products of I–III as revealed by HPLC analysis using the systems described previously (Bundgaard and Buur, 1987; Bundgaard and Nielsen, 1987). As also described in these previous works the *N*-hydroxyalkyl derivatives in a subsequent slower step were observed to hydrolyze to give the corre-

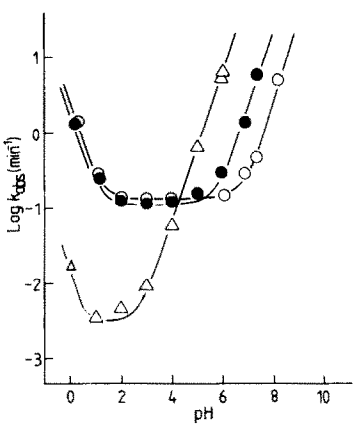


Fig. 3. The pH–rate profiles for the decomposition of the aspirin esters I (○), II (●) and III (△) in aqueous solution ($\mu = 0.5$) at 37 °C.

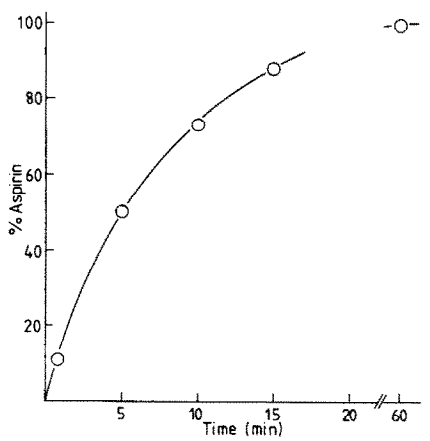
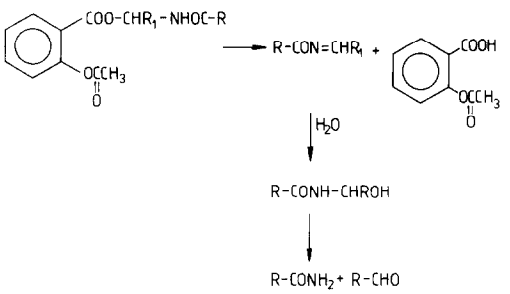


Fig. 4. Time course for aspirin formed upon degradation of the ester I in aqueous solution of pH 5.00 at 37 °C.



Scheme 1

sponding amide (benzamide or acetamide). For example, *N*-(hydroxymethyl)benzamide formed upon hydrolysis of compound II decomposes with a half-life of 160 h at pH 7.4 and 37 °C whereas α-hydroxy-benzoylglycine benzyl ester formed from compound III shows a half-life of 4.5 h at the same conditions (Bundgaard and Buur, 1987).

According to our previous study (Bundgaard and Nielsen, 1987) on similar compounds the most likely mechanism responsible for the facile decomposition of the aspirin esters I–III is a unimolecular elimination–addition process, which can be regarded as an S_N1 reaction, with the formation of a transient *N*-acylimine intermediate as depicted in Scheme 1. In this mechanism the rate-determining step involves elimination of carboxylate anion to give an *N*-acylimine intermediate which in a subsequent fast step undergoes attack by a solvent (water) molecule, giving the α-hydroxyalkyl amide.

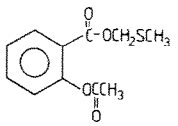
Considering the relative reactivities of the esters I–III the k_{OH}-value for ester III is considerably higher than those for compounds I and II. This higher reactivity may be ascribed to the large polar effects of an ester and amide group, rendering the *N*-acylimine formation easier. The reason for the much lower reactivity of compound III in acidic solution as expressed by the constants k_H and k_0 is not, on the other hand, evident but the steric differences in the alcohol parts of ester III and esters I and II may probably be a factor of importance.

Hydrolysis in plasma solutions

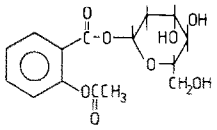
The rates and products of decomposition of compounds I–III were determined in 80% human plasma (pH 7.4) solutions at 37 °C. According to the decomposition mechanism proposed no enzymatic catalysis of the ester cleavage to yield aspirin should be expected. However, an enzymatically catalyzed hydrolysis of the acetyl ester moiety to yield the corresponding salicylate ester derivative along the k_2-route (Fig. 1) could compete with the non-enzymatic k_1-route and thus diminish the amounts of aspirin to be formed from the esters. In the case of compounds II and III a quantitative yield of aspirin was observed in plasma solutions (Table 3). The half-lives of degradation of these compounds at pH 7.4 and 37 °C are only 8 s (II) and 0.3 s (III) so any plasma-catalyzed cleavage of the acetyl ester moieties in these compounds does not significantly compete with the spontaneous decomposition leading to free aspirin. The ester I exhibits a half-life of spontaneous hydrolysis of 1.1 min at pH 7.40 and 37 °C and in this case, the plasma catalyzed hydrolysis of the acetyl ester moiety competes to some extent with the spontaneous decomposition since the amount of aspirin formed from this compound amounts to 80% in 80% human plasma solutions.

Assessment of the esters I–III as aspirin prodrugs

The data presented show that aspirin esters of *N*-(hydroxyalkyl)amides such as compounds I–III behave as true aspirin prodrugs, i.e., they hydrolyze in aqueous solution as well as in the presence of plasma with regeneration of the parent aspirin in stoichiometric amounts, for compound I in amounts of 80%. Compounds I and II show a half-life of decomposition of 5–6 min at pH 2–5.5 but are more unstable at both more acidic and basic pH values. Compound III is extremely rapidly hydrolyzed at pH > 5 but is more stable than the esters I and II at lower pH values. Thus, in the pH-range 1–3 corresponding to the normal gastric pH range ester III is hydrolyzed with a half-life of 70–200 min at 37 °C (cf. Table 1). This relatively high acid stability implies that the compound to a significant extent may pass unhydrolyzed though the stomach upon oral administration. Once present in the intestine in dissolved form the compound should hydrolyze immediately with formation of aspirin.



IV



V

Previously studied putative aspirin prodrugs which regenerate the parent aspirin by a non-enzymatic reaction in aqueous solution include the methylthiomethyl ester IV (Loftsson et al., 1981) and the deoxyglucose acylal V (Hussain et al., 1979). The ester IV is hydrolyzed via a unimolecular alkyl–oxygen cleavage mechanism with a pH-independent half-life of 50 min at pH 2–9 and 37 °C (Loftsson and Bodor, 1981). In the presence of plasma, however, enzymatic deacetylation predominates greatly over the methylthiomethyl ester hydrolysis so that less than 5–10% aspirin is formed (Loftsson et al., 1981). The acylal derivative V also undergoes a pH-independent hydrolysis in the pH-range 3–9, the half-life being 7 min at 37 °C (Hussain et al., 1979). The hydrolysis of this compound has not been studied in the presence of plasma but also in this case, an enzymatic deacetylation is expected to make a significant contribution to the overall rate of degradation and thus diminish the aspirin formation.

Compound III combines the properties of being reasonably acid-stable with a high susceptibility to be hydrolyzed to aspirin at pH values greater than 5. Such a compound could therefore be a potentially useful aspirin prodrug for oral administration. Compound III is derived from α-hydroxy-*N*-benzoylglycine benzyl ester but based on previous kinetic results of analogous derivatives (Bundgaard and Buur, 1987) it may be readily feasible to build into the molecule other groups than the benzyl ester moiety in order to optimize the physiochemical properties and still maintain the combination of adequate acid stability and lability at neutral pH. Thus, structures containing an amide or another ester with a solubilizing functionality like an amino, hydroxyl or carboxyl group can readily be imagined.

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