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Prodrugs as drug delivery systems. 44. O-Acyloxymethyl, O-acyl and N-acyl salicylamide derivatives as possible prodrugs for salicylamide

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Summary

The hydrolysis kinetics and bioactivation characteristics of various O-acyloxymethyl, O-acyl and N-acyl derivatives of salicylamide were studied to assess their suitability as prodrugs for the parent compound in an effort to increase its systemic availability following oral or rectal administration. The O-acyloxymethyl derivatives were found to be much more stable in aqueous solution than the corresponding simple O-acylsalicylamides as they, in contrast to the latter, do not undergo an intramolecular O–N acyl migration to any significant extent. The rate of hydrolysis of the O-acyloxymethyl derivatives to give salicylamide was markedly accelerated in human plasma, the half-life of hydrolysis in 80% plasma solutions at 37°C being 16 min, 5.5 min and 2.7 h for the O-acetoxymethyl, O-butyryloxymethyl and O-pivaloyloxymethyl derivatives, respectively. The half-lives of hydrolysis of the corresponding O-acyl derivatives were less than 1 min at the same conditions whereas that of N-acetylsalicylamide was 3.6 h. It is suggested that O-acyloxymethylation of salicylamide may be a potentially useful approach to improve the oral or rectal delivery characteristics of the drug by affording a protection of the phenolic hydroxyl group against metabolic conjugation reactions. O-(butyryloxymethyl)salicylamide was found to possess both a higher water solubility and lipophilicity than salicylamide which was attributed to a decreased crystal lattice energy as manifested by the lower melting point of the derivative.

Introduction

Salicylamide (I) has analgesic, antipyretic and hypnotic activities but its clinical value is limited due to extensive presystemic biotransformation after oral as well as rectal administration (Levy, 1965; Levy and Matsuzawa, 1967; Barr and Riegelman, 1970; Gugler et al., 1975; Fleckenstein et al., 1976; Shibasaki et al., 1981; De Boer et al., 1983; Iwamoto et al., 1983). Since the first-pass metabolism primarily involves conjugation of the phenolic group with glucuronic acid and sulfuric acid (Morris and Levy, 1983), protection of this group may possibly minimize the metabolic inactivation and hence increase the oral or rectal bioavailability. To be useful as prodrugs such transient salicylamide derivatives should possess adequate water and lipid solubilities in order to ensure satisfactory absorption and furthermore, they should exhibit a stability which makes the

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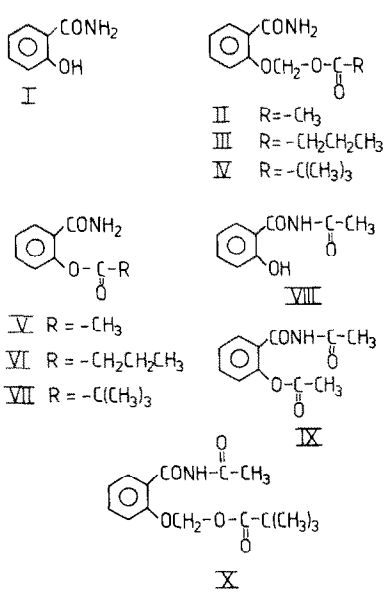
derivatives resistant to undergo first-pass metabolism, yet allowing them to be cleaved to the parent drug after entrance in the systemic circulation.

Previous attempts to develop prodrugs of salicylamide have concerned O-acetyl, O-acetylsalicyloyl and O-glutaryl esters (Babhair and Hussain, 1983). These derivatives were found to be very easily cleaved enzymatically to salicylamide but investigations of their absorption behaviour in dogs revealed only a marginal improvement in the oral bioavailability of the compounds relative to salicylamide (Babhair, 1979). Presumably, these salicylamide esters are too labile to resist first-pass hydrolysis.

In the present work various O-acyloxymethyl derivatives (II–IV) of salicylamide were prepared and evaluated as possible prodrug forms. These compounds were thought to be more resistant towards enzymatic hydrolysis than esters obtained by direct acylation of the phenolic hydroxyl group in salicylamide. Therefore, the corresponding O-acylated salicylamide derivatives (V–VII) were also prepared and studied. Furthermore, N-acetylsalicylamide (VIII), O,N-diacetylsalicylamide (IX) and N-acetyl-O-(pivaloyloxymethyl)salicylamide (X) were included in the study. In this paper, the chemical- and enzyme-mediated conversion of the derivatives is described along with data on the aqueous solubility and lipophilicity of some of the compounds.



Formulae I-X

Materials and Methods

Apparatus

Ultraviolet spectral measurements were performed with a Shimadzu UV-190 spectrophotometer equipped with a thermostatically controlled cell compartment, using 1-cm quartz cells. ^{1}H-NMR spectra were run on a Varian 360L instrument. 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 uncorrected. High-performance liquid chromatography (HPLC) was done with a Spectra-Physics Model 3500B instrument equipped with a variable wavelength detector, a 10-μl loop injection valve and a column (250 × 4 mm) packed with LiChrosorb RP-8 (7 μm) particles) (E. Merck, Darmstadt). Some HPLC measurements were also performed with a Kontron apparatus consisting of an LC Pump T-414, a Uvikon 740LC UV detector, a 20-μl loop injection valve and a Chrompack column (100 × 3 mm) packed with CP Spher C8 (8 μm particles). Microanalyses were performed by G. Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, Ballerup, Denmark.

Chemicals

Salicylamide was obtained from E. Merck, Darmstadt. Chloromethyl pivalate was purchased from Fluka AG, Switzerland. Chloromethyl acetate and chloromethyl butyrate were prepared as described by Ulich and Adams (1921). Gravity column chromatography (CC) was performed using silica gel (Woelm, 0.063–0.200 mm).

Synthesis of salicylamide derivatives (II–X)

The compounds II–IV and X were prepared by reaction of salicylamide or N-acetylsalicylamide with an iodomethyl ester prepared in situ by treatment of the corresponding chloromethyl ester with sodium iodide in acetone (cf. Bodor et al., 1983).

General procedure

O-(Acetoxymethyl)salicylamide (II). A mixture of salicylamide (1.0 g; 7.3 mmol) and potassium carbonate (2.02 g; 14.6 mmol) in acetone (40 ml) was stirred at room temperature for 30 min. In a separate flask chloromethyl acetate (1.19 g; 11 mmol) and dry potassium iodide (1.98 g; 13.2 mmol) in acetone (40 ml) were reacted at room temperature for 30 min. The iodomethyl acetate was transferred by decantation to the flask containing salicylamide and potassium carbonate. The mixture was refluxed for 4 h, filtered, and the filtrate was evaporated in vacuo. Ethyl acetate (60 ml) was added to the residue and the mixture was washed twice with water (50 ml). The dried ethyl acetate solution was evaporated in vacuo. After CC (silica gel; eluent: toluene–ethyl acetate (1 : 1) containing 1% of acetic acid) of the residue the title compound was isolated and recrystallized from chloroform–petroleum ether. Yield: 378 mg (25%), m.p. 92–93°C. ^{1}H NMR ($CDCl_3$)δ : 2.05 (s, 3H); 5.88 (s, 2H); 7.0–7.8 (m, 3H); 8.1 (q, 1H). *Anal.*: Calculated for $C_{10}H_{11}NO_4$: C, 57.41; H, 5.30; N, 6.70%. Found: C, 57.37; H, 5.26; N, 6.66%.

O-(Butyryloxymethyl)salicylamide (III). From salicylamide (2.0 g; 14.6 mmol) and chloromethyl butyrate (3.0 g; 21.9 mmol). Yield: 1.63 g (47%), m.p. 56–58°C (from ether–petroleum ether). ^{1}H NMR ($CDCl_3$)δ : 0.92 (t, 3H); 1.61 (m, 2H); 2.33 (t, 2H); 5.93 (s, 2H); 7.0–7.7 (m, 3H); 8.22 (q, 1H). *Anal.*: Calculated for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.90%. Found: C, 60.74; H, 6.38; N, 5.93%.

O-(Pivaloyloxymethyl)salicylamide (IV). From salicylamide (1.0 g, 7.3 mmol) and chloromethyl pivalate (1.66 g; 11 mmol). Yield: 532 mg (29%), m.p. 94–96°C (from ether–petroleum ether). ^{1}H NMR ($CDCl_3$)δ : 1.20 (s, 9H); 5.93 (s, 2H); 7.0–7.75 (m, 3H); 8.28 (q, 1H). *Anal.*: Calculated for $C_{13}H_{17}NO_4$: C, 62.14; H, 6.82; N, 5.58%. Found: C, 62.25; H, 6.83; N, 5.61%.

N-Acetyl-O-(pivaloyloxymethyl)salicylamide (X). From N-acetylsalicylamide (0.8 g; 4.5 mmol) and chloromethyl pivalate (1.0 g; 6.7 mmol). The compound could be isolated without CC by recrystallization from ether–petroleum ether. Yield: 813 mg (62%), m.p. 56–58°C. ^{1}H NMR ($CDCl_3$)δ : 1.18 (s, 9H); 2.57 (s, 3H); 5.98 (s, 2H); 7.0–7.7 (m, 3H); 8.18 (q, 1H). *Anal.*: Calculated for $C_{15}H_{19}NO_5$: C, 61.42; H, 6.53; N, 4.78%. Found: C, 61.30; H, 6.55; N, 4.71%.

O-Acyl derivatives of salicylamide (V–VII). These compounds were prepared by dissolving salicylamide (4.1 g; 0.3 mol) in a mixture of pyridine (3–8 ml) and the appropriate acid anhydride (0.06 mol) according to the method described by Gordon (1967). After standing for 5–10 min at room temperature the salicylamide esters precipitated or ether was added to precipitate the compounds. The compounds were recrystallized from ethyl acetate, m.p. 144–145°C (O-acetylsalicylamide (V)), reported m.p. 144–145°C (Gordon, 1967); m.p. 101–102°C (O-butyrylsalicylamide (VI)). *Anal.* (VI): Calculated for $C_{11}H_{13}NO_3$: C, 63.75; H, 6.32; N, 6.76%. Found: C, 63.54; H, 6.40; N, 6.67%. The O-pivaloylsalicylamide (VII) obtained was contaminated with 10% salicylamide as determined by HPLC.

N-Acetylsalicylamide (VIII) was prepared by refluxing a 10% solution of compound V in methanol for 5 h. After evaporation of the solvent, the solid residue was recrystallized from ethyl acetate, m.p. 140–142°C, reported m.p. 141.5–143°C (Gordon, 1967).

O,N-Diacetylsalicylamide (IX) was prepared by refluxing a solution of 1.8 g of O-acetylsalicylamide in 5 ml of acetic anhydride for 8 h. The solution was evaporated in vacuo and the residue allowed to crystallize by standing at 4°C for 48 h, m.p. 63–64°C (from ether–petroleum ether); reported m.p. 64–65.5°C (Gordon, 1967), 67–68°C (Titherley and Hicks, 1911).

Kinetic measurements

The degradation of the salicylamide derivatives was studied 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 were followed by using reversed-phase HPLC procedures. Mobile phase systems of 30–65% v/v methanol in 0.01 M acetate

buffer of pH 5.0 were used, the concentration of methanol being adjusted for each compound to give an appropriate retention time (2–15 min). In most cases the solvent systems used allowed quantitation of all degradation products formed simultaneously with quantitation of the starting material. The column effluent was monitored at 230 nm (Spectra-Physics HPLC Instrument) or 213 nm (Kontron Instrument). 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 100–200 μl of a stock solution of the compounds in ethanol or acetonitrile to 10 ml of pre-heated buffer solution in screw-capped test tubes, the final concentration of the compounds being about 10^{-3} M. The solutions were kept in a water-bath at 37°C and at appropriate intervals samples were taken and chromatographed. Pseudo-first-order rate constants for the degradation were determined from the slopes of linear plots of the logarithm of residual salicylamide derivative against time.

In case of the hydrolysis studies performed in human plasma diluted to 80% with 0.05 M phosphate buffer of pH 7.40, samples of 250 μl were withdrawn at appropriate intervals and added to 1000 μl of ethanol in order to deproteinize the plasma. After immediate mixing and centrifugation for 2–3 min, 10 or 20 μl of the clear supernatant was analyzed by HPLC as described above.

The degradation of the compounds II–IV and V–VIII was also followed spectrophotometrically in some cases by monitoring the absorbance increase at 327 nm and 355 nm, respectively. The reactions were performed in 2.5 ml aliquots portions of buffer solutions in a thermostated quartz cuvette and were initiated by adding 100 μl of stock solutions of the derivatives in acetonitrile to give a final concentration of about 2×10^{-4} M. Pseudo-first-order rate constants were calculated from the slopes of linear plots of log $(A_\infty - A_t)$ against time where A_∞ and A_t are the absorbance readings at infinity and time t, respectively.

Determination of aqueous solubility and partition coefficients

The aqueous solubility of some derivatives was determined at 23°C by adding excess amounts to 0.05 M acetate buffer of pH 4.0 and allowing the suspensions to rotate on a mechanical spindle for 24 h after being placed in an ultrasonic water-bath for 30 min. The concentration of the compounds in the saturated solutions was calculated from the measured peak heights by reference to those of standards chromatographed under the same conditions.

Partition coefficients were determined in an octanol–0.05 M phosphate buffer (pH 7.40) system as previously described (Bundgaard et al., 1986).

Results and Discussion

Hydrolysis of the derivatives II–IV in buffer solutions

The kinetics of hydrolytic breakdown of the O-acyloxymethyl salicylamide derivatives II–IV was studied in aqueous solution at 37°C in the pH range 1–11.6. At constant pH and temperature the disappearance of the derivatives followed strict first-order kinetics over several half-lives as determined by HPLC. In some cases (pH 9–10) the rate of a given reaction was determined using both the direct UV-spectrophotometric method and the HPLC method and the values of the rate constants obtained therefrom agreed within ± 3%. 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 degradation of the derivatives II–IV is shown in Fig. 1 where the logarithm of the observed pseudo-first-order rate constants (k) is plotted against pH. The effect of pH upon the hydrolysis of the O-acetoxymethyl derivative (II) was examined in detail over the pH range 1–10. As seen from Fig. 1 the pH–rate profile for this derivative is U-shaped, indicating the occurrence of specific acid and base catalysis as well as a spontaneous or water-catalyzed reaction according to the following rate expression:

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

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

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

Values of the second-order rate constants for the specific acid (k_H) and specific base (k_{OH}) catalyzed hydrolysis were determined from the straight line portions of the pH–rate profiles at low and high pH values, respectively, whereas the value of the first-order rate constant for spontaneous hydrolysis (k_0) was obtained on the basis of Eqn. 1. The values of the rate constants derived are listed in Table 1. In Fig. 1 the solid curves or lines drawn were constructed from these constants and Eqn. 1.

Inspection of the rate data shows that the pivaloyloxymethyl derivative IV is considerably more stable than the butyryloxymethyl and acetoxymethyl derivatives. The polar effects of the acyl groups in these compounds are almost identical and the differences in reactivity can solely be ascribed to differences in the steric properties as shown in Fig. 2, where log k_H and log k_{OH} are plotted against Charton's steric parameter ν (Charton, 1977).

TABLE 1

RATE DATA FOR THE HYDROLYSIS OF VARIOUS O-ACYLOXYMETHYL DERIVATIVES OF SALICYLAMIDE IN AQUEOUS SOLUTION AT 37°C AND $\mu = 0.5$

Compound	k_H ($M^{-1} \cdot min^{-1}$)	k_0 (min^{-1})	k_{OH} ($M^{-1} \cdot min^{-1}$)
II	0.023	1.5×10^{-5}	417
III	0.018	–	195
IV	0.0068	–	15.1

The disappearance of the derivatives II–IV was found to be accompanied by the formation of salicylamide in 97–100% yields as evidenced by HPLC analysis. A typical time-course of the formation of salicylamide is shown in Fig. 3. No induction period in the formation of salicylamide is apparent, the rate of formation following strict first-order kinetics at constant pH. The hydrolysis of the derivatives most likely takes place via a two-step reaction as depicted in Scheme 1. The rate-determining step is cleavage of the ester grouping, resulting in the formation of a hemiacetal which instantaneously decomposes to salicylamide and formaldehyde. This reaction scheme is similar to that proposed for the hydrolysis of

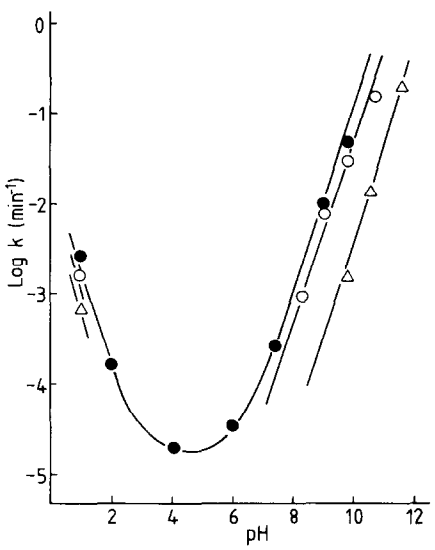


Fig. 1. The pH–rate profiles for the degradation of compound II (●), compound III (○) and compound IV (△) in aqueous solution ($\mu = 0.5$) at 37°C.

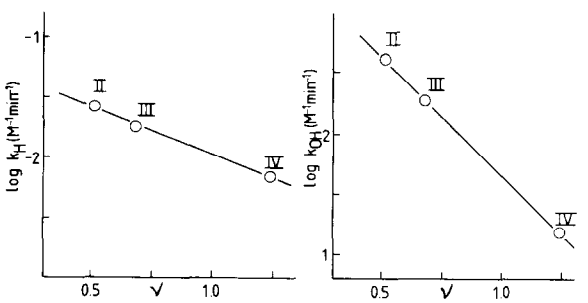


Fig. 2. Plots of log k_H and log k_{OH} vs the steric substituent parameter ν for the O-acyloxymethyl salicylamide derivatives II–IV. The ν values, taken from Charton (1977), refer to the alkyl moieties in the acyl groups, i.e. methyl, propyl and tertiary butyl.

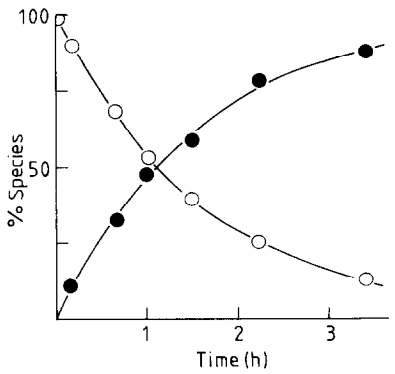


Fig. 3. Time-courses for compound II (○) and salicylamide (●) in the degradation of compound II at pH 9.01 (at 37°C).

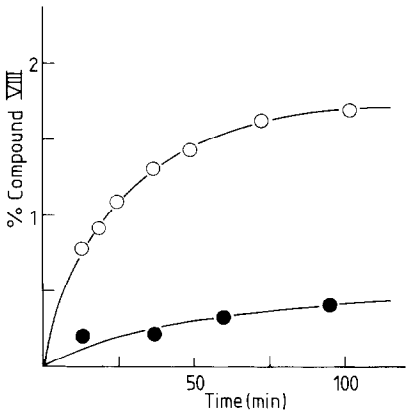
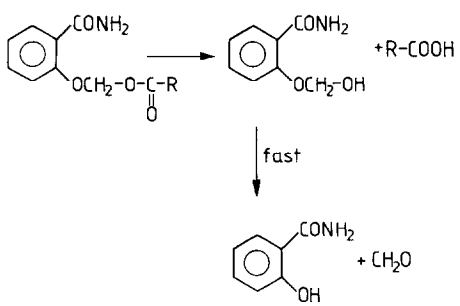


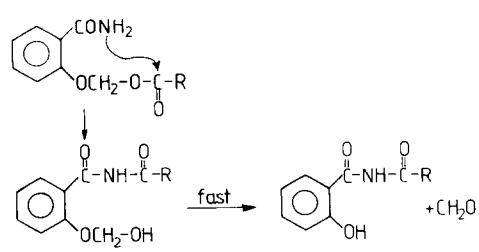
Fig. 4. Plots showing the formation of N-acetylsalicylamide (VIII) during degradation of O-(acetoxymethyl)salicylamide (II) at pH 9.80 (○) and pH 9.01 (●) at 37°C.

other aryl acylals (Fife and De, 1974; McClelland, 1975; Loftsson and Bodor, 1982; Bodor et al., 1983).



Scheme 1

In neutral and alkaline solution a minor reaction pathway of the compounds leading to the formation of the corresponding N-acyl salicylamide derivative was observed to occur. The N-acylated derivatives arising from II and III were identified on the basis of their HPLC retention behaviour in comparison with those of authentic samples and by showing that these products degraded slowly into salicylamide following their formation. The amount of N-acyl salicylamide formed was found to be about 1% in neutral and alkaline solutions whereas no formation was detected at pH < 6. Thus, N-acetylsalicylamide was formed in amounts of 1.7% at pH 9.80, 0.5% at pH 9.01, 0.9% at pH 7.40 and about 0.2% at pH 6.00 upon degradation of O-(acetoxymethyl)salicylamide (Fig. 4). The slightly varying values at pH 7.4–9.8 may be ascribed to experimental reasons. Taking the hydrolysis of the N-acyl derivatives following their formation into account the total yield of the compounds may be estimated to be about 2%. The formation of the N-acyl derivatives most likely takes place via an intramolecular O → N acyl transfer reaction (Scheme 2) in analogy with the behaviour of O-acyl salicylamides (see below). The amide anion is thought to be the species attacking the the ester carbonyl group (cf. Behme and Cordes, 1964), implying apparent hydroxide ion catalysis of the reaction at pH values below the pK_a of the amide (about 14–15). This is in accord with the findings of formation of N-acyl salicylamide in both neutral and alkaline solution.



Scheme 2

Enzymatic hydrolysis of compounds II–IV

The susceptibility of the O-acyloxymethyl de-

rivatives to undergo a potential enzymatic hydrolysis was studied at 37°C in 0.02 M phosphate buffer solutions (pH 7.4) containing 80% human plasma. The hydrolysis of the derivatives followed strict first-order kinetics at the experimental conditions used (Fig. 5) and proceeded in all cases to give salicylamide in quantitative amounts. No formation (i.e. < 0.2%) of N-acylated salicylamide was observed.

As appears from the rate data obtained (Table 2) plasma enzymes markedly accelerate the rate of hydrolysis, the butyryloxymethyl derivative (III) exhibiting the shortest half-life in plasma. The lower reactivity of compound IV may be ascribed to steric hindrance exhibited by the bulky pivaloyl group. The order and magnitude of reactivity is similar to that observed previously for the plasma-catalyzed hydrolysis of morpholine N-Mannich bases of compounds II–IV (Bundgaard et al., 1986).

Hydrolysis of O-acyl salicylamide derivatives

The degradation of O-acetyl-, O-butyryl- and O-pivaloylsalicylamide (V–VII) was studied in aqueous solutions at pH 6.9–8.3 (37°C). In accordance with earlier studies on O-acetylsalicylamide (Behme and Cordes, 1964; Babhair and Hussain, 1983) the compounds were found to undergo a rapid O → N acyl transfer reaction resulting in the formation of the corresponding N-acylsalicylamide derivative (Scheme 3). HPLC analysis of the reaction solution revealed a 100% transformation to the N-acylsalicylamides with no formation (i.e. < 0.2%) of salicylamide. The pseudo-first-order rate constants for the intramolecular reaction increase linearly with hydroxide ion concentration in the pH range 6.8–8.3 as seen in Fig. 6. This behaviour is consistent with a mechanism involving nucleophilic attack of the amide anion on the ester carbonyl group as shown in Scheme 3 (Behme and Cordes, 1964; Gordon, 1967; Russell and Topping, 1975; Babhair and Hussain, 1983). The calculated apparent hydroxide-ion catalytic rate constants for the reactions of compounds V–VII are listed in Table 3.

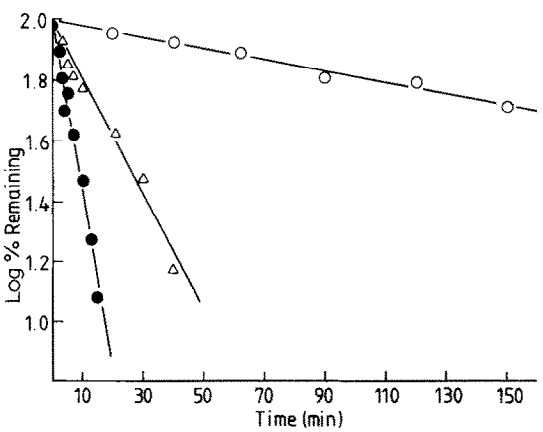


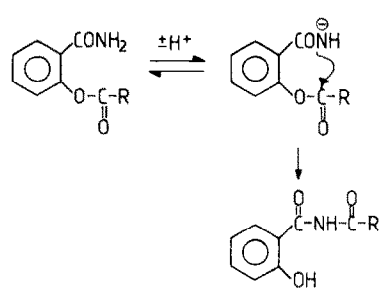
Fig. 5. First-order plots for the hydrolysis of the O-acyloxymethyl salicylamide derivatives II (△), III (●) and IV (○) in 80% human plasma solutions (pH 7.40) at 37°C.

TABLE 2

HALF-LIVES FOR THE HYDROLYSIS OF VARIOUS SALICYLAMIDE DERIVATIVES IN AQUEOUS BUFFER SOLUTION AND IN 80% HUMAN PLASMA (pH 7.4) AT 37°C

Compounds	Half-lives	
	pH 7.4 buffer	80% plasma
O-(Acetoxymethyl)salicylamide (II)	46 h	16 min
O-(Butyryloxymethyl)salicylamide (III)	98 h [a]	5.5 min
O-(Pivaloyloxymethyl)salicylamide (IV)	1270 h [a]	2.7 h

[a] Estimated from the rate data obtained at pH > 8.



Scheme 3

In the presence of 80% human plasma (pH 7.4, 37°C) O-acetylsalicylamide (V) and O-butyrylsalicylamide (VI) were found to cleave predominantly (> 80%) to salicylamide and not to rearrange to the imide. In the case of O-pivaloylsalicylamide (VII), however, this enzyme-catalyzed

TABLE 3

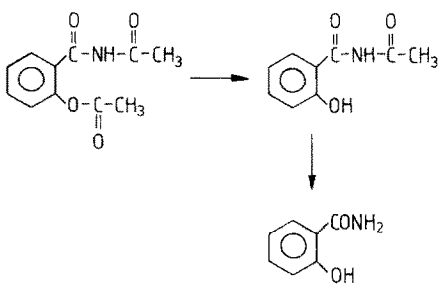
RATE DATA FOR THE OVERALL DEGRADATION (HYDROLYSIS OR REARRANGEMENT) OF VARIOUS SALICYLAMIDE DERIVATIVES AT 37°C

Compound	k_{OH} ($M^{-1} \cdot min^{-1}$)	Half-lives	
		pH 7.4 buffer	80% human plasma
O-Acetylsalicylamide (V)	18.2×10^5 [a]	0.6 min	0.3 min
O-Butyrylsalicylamide (VI)	11.0×10^5	1.0 min	0.2 min
O-Pivaloylsalicylamide (VII)	4.0×10^5	2.9 min	0.9 min
O,N-Diacetylsalicylamide (IX)	2.1×10^3	9.3 h	0.4 min
N-Acetylsalicylamide (VIII)	–	67 h	3.6 h
N-Acetyl-O-(pivaloyloxymethyl)salicylamide (X)	425	45 h	3.8 h

[a] A k_{OH} value of 8.4×10^5 $M^{-1} \cdot min^{-1}$ was reported at 25°C and $\mu = 1.0$ (Babhair and Hussain, 1983).

hydrolysis of the ester moiety could not compete fully with the intramolecular N-acylsalicylamide formation. In 80% plasma solutions N-pivaloylsalicylamide was the major product. Apparently, the bulky pivaloyl group exhibits greater steric hindrance against enzymatic hydrolysis than against the O → N acyl transfer reaction. The rate data obtained in the plasma solutions are given in Table 3.

The hydrolytic behaviour of O,N-diacetylsalicylamide (IX) was examined in buffer solutions of pH 7.4–8.3 as well as in 80% human plasma solution. Both in buffer solutions and in the presence of plasma the compound was found to cleave exclusively to N-acetylsalicylamide which subsequently showed a slow degradation to give salicylamide (Scheme 4). The rate data obtained are listed in Table 3. As can be seen from the data, the O,N-diacetyl derivative is much more stable in aqueous solution than the O-acetyl derivative as a consequence of its reluctance to undergo an intramolecular O → N acyl transfer reaction.



Scheme 4

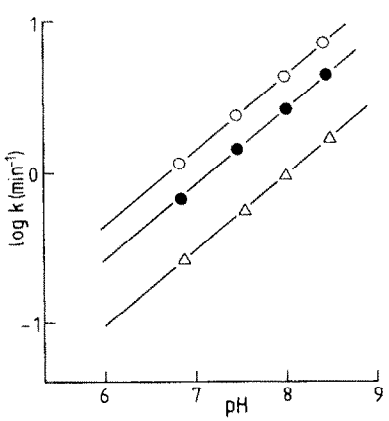


Fig. 6. The pH–rate profiles for the conversion of compound V (○), compound VI (●) and compound VII (△) to the corresponding N-acyl salicylamides in aqueous solution ($\mu = 0.5$) at 37°C.

Hydrolysis of N-acetylsalicylamide (VIII) and N-acetyl-O-(pivaloyloxymethyl)salicylamide (X)

So far imides formed by acylation of simple amides have not been considered as potential prodrug forms. It was, however, found that N-acetylsalicylamide was relatively rapidly cleaved to salicylamide and acetic acid in the presence of 80% human plasma (pH 7.4, 37°C) (Fig. 7). The hydrolysis exhibited strict first-order kinetics with a half-life of 3.6 h. In the absence of plasma, i.e. in 0.02 M phosphate buffer of pH 7.4 and at 37°C, the half-life of hydrolysis of N-acetylsalicylamide to give salicylamide (> 95%) was determined to be 67 h, thus demonstrating the enzymatic activity of plasma in catalyzing the N-acylamide hydrolysis.

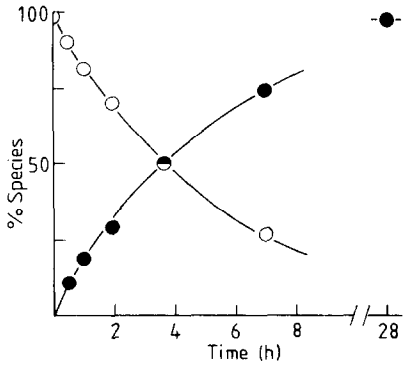


Fig. 7. Plots showing the rate of hydrolysis of N-acetylsalicylamide (○) in 80% human plasma (37°C) and the formation of salicylamide (●) as determined by HPLC.

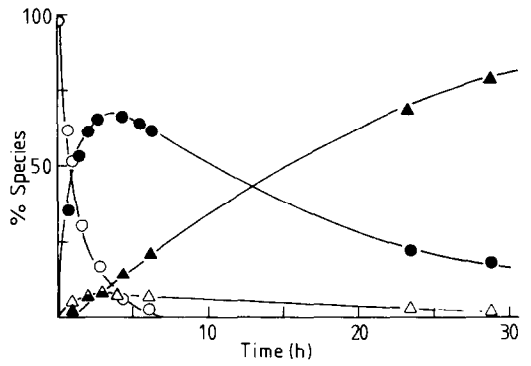
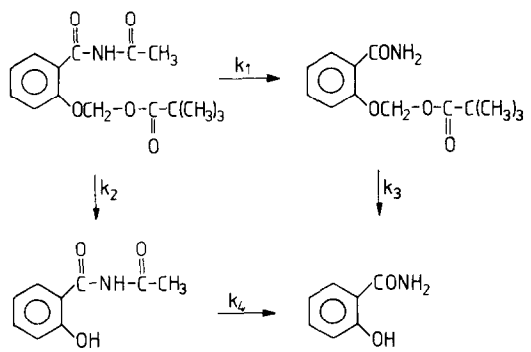


Fig. 8. Time-courses for compound X (○), O-(pivaloyloxymethyl)salicylamide (●), N-acetylsalicylamide (△) and salicylamide (▲) in the degradation of compound X at pH 9.41 and 37°C. The concentrations at various times, expressed as percent of the initial concentration of X, were determined by an HPLC procedure enabling separation of all compounds (mobile phase: 0.01 M acetate pH 5.0–methanol 3:2 v/v).

Several other N-acyl derivatives of salicylamide as well as of other amides were found to behave in a similar way. The results of a more detailed study of these compounds will be described in a later report. It is of interest to note that N-acetylsalicylamide several years ago was shown to be completely transformed to salicylamide and metabolites thereof in man after oral administration (Rayet et al., 1951).

Since the N-acetylsalicylamide is susceptible to undergo enzymatic hydrolysis and thus to function as a prodrug of salicylamide it was found of interest to study an O-acyloxymethyl derivative of it, considering the need to protect the phenolic OH-group against metabolic conjugation reactions. The O-pivaloyloxymethyl derivative (X) was prepared and its hydrolytic decomposition studied. In neutral and alkaline solutions (pH 7.4–9.4) the rate of hydrolysis showed specific base catalysis (Table 3). The disappearance of X was found to be accompanied by the formation of O-(pivaloyloxymethyl)salicylamide (IV) and N-acetylsalicylamide (VIII), identified on the basis of their HPLC retention behaviour in comparison with those of authentic samples. Following their formation these products degraded into salicylamide. This final product of hydrolysis was formed in quantitative amounts (> 95%) at all pH values studied (pH 7.4–9.4) as evidenced by HPLC analysis of completed reaction solutions. The time-courses of the various species are shown in Fig. 8 from a run performed at pH 9.41. The proposed hydrolysis reactions taking place are depicted in Scheme 5, where k_1–k_4 are pseudo-first-order rate constants for the depicted reactions. Product analysis showed that the k_1-reaction involving cleavage of the imide moiety is the dominating degradation



Scheme 5

route in neutral and alkaline solution, accounting for about 90% of the total degradation.

As was found for the individual compounds, N-acetylsalicylamide (VIII) and O-(pivaloyloxymethyl)salicylamide (IV), the hydrolysis of the combined derivative X was subject to marked enzymatic catalysis by human plasma. The half-life for the overall degradation of the compound X in 80% human plasma was 3.8 h, the half-life in buffer solution without plasma being 45 h. From the degradation course observed in the plasma

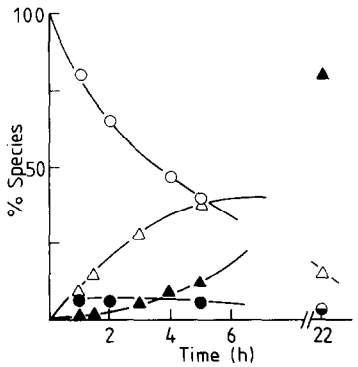


Fig. 9. Time-course for compound X (○), O-(pivaloyloxymethyl)salicylamide (●), N-acetylsalicylamide (△) and salicylamide (▲) in the degradation of compound X in 80% human plasma and 37°C. The concentrations at various times, expressed as percent of the initial concentrations of X, were determined by an HPLC procedure enabling separation of all compounds (mobile phase: 0.01 M acetate pH 5.0–methanol 3:2 v/v).

solution (Fig. 9) it can be seen that the k_1- and k_2-reactions (cf. Scheme 5) are of about the same importance in this case.

Lipophilicity and aqueous solubility of the O-acyloxymethyl derivatives

Partition coefficients (P) for the O-acyloxymethyl derivatives as determined using an octanol–aqueous buffer system (pH 7.4) are listed in Table 4 along with melting points and solubilities in 0.05 M acetate buffer of pH 4.0. The difference in the log P values for the derivatives II–IV is as expected on the basis of the lipophilic π substituent values (Hansch and Leo, 1979). An increase in lipophilicity is generally accompanied by a decrease in water solubility. Inspection of the data in Table 4 reveals, however, that the water solubility of the O-butyryloxymethyl derivative III is in fact increased in comparison to that of salicylamide despite its higher log P value. This behaviour may be attributed to a decreased crystal lattice energy in compound III relative to salicylamide as manifested in the lower melting point of III. Salicylamide may exhibit both intra- and intermolecular hydrogen bonding in the crystal lattice and blocking of the phenolic hydroxyl group may conceivably lead to decreased hydrogen bonding and hence decreased crystal lattice energy.

TABLE 4

MELTING POINTS, PARTITION COEFFICIENTS (P) AND AQUEOUS SOLUBILITIES OF SALICYLAMIDE AND VARIOUS O-ACYLOXYMETHYL DERIVATIVES

Compound	m.p. (°C)	log P [a]	S [b] (mg·ml^{-1})
Salicylamide (I)	140	1.15 [c]	2.2
O-(Acetoxymethyl) salicylamide (II)	92–93	0.52	> 5 [d]
O-(Butyryloxymethyl) salicylamide (III)	56–58	1.66	2.5
O-(Pivaloyloxymethyl) salicylamide (IV)	94–96	2.00	0.61

[a] Determined in octanol–phosphate buffer of pH 7.4 at 23°C.
[b] Solubility in acetate buffer of pH 4.0 at 23°C.
[c] Log P = 1.24 for undissociated salicylamide (pK_a = 8.1).
[d] A sample of 10 mg was fully dissolved in 2 ml of buffer; no further samples were available.

Conclusions

The results obtained suggest that O-acyloxymethyl derivatives of salicylamide may be potentially useful prodrug forms. The derivatives are cleaved quantitatively to salicylamide in human plasma and by appropriate selection of the acyl group it is feasible to obtain derivatives possessing varying enzymatic lability. The derivatives are more stable towards enzymatic hydrolysis than the corresponding O-acyl derivatives and hence should be able to protect salicylamide against first-pass metabolism to a higher extent. Furthermore, the O-acyloxymethyl derivatives are much more stable in aqueous solution than the simple O-acylsalicylamides because of the facile intramolecular O → N acyl transfer reactions of the latter compounds. Besides cleavage rates, such physicochemical properties as aqueous solubility and lipophilicity can be modified by appropriate selection of the acyl group in the acyloxymethyl derivatives. As has been demonstrated it is feasible to obtain derivatives possessing higher lipophilicity than the parent drug and at the same time also higher water solubility. In this respect it should be noted that derivatization of the prodrug forms by N-acylation as exemplified with the derivative X or by N-Mannich base formation within the amide

moiety (Bundgaard et al., 1986) can be used to further modify the lipophilicity and solubility characteristics. Studies are in progress to determine the oral and rectal bioavailability of some of the derivatives.

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