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Prodrugs as drug delivery systems. 43. O-Acyloxymethyl salicylamide N-Mannich bases as double prodrug forms for amines

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Summary

Due to their rapid cleavage at physiological pH salicylamide N-Mannich bases have been suggested as prodrug forms for primary and secondary amines. A drawback of the Mannich bases is, on the other hand, their limited stability in vitro, raising some stability-formulation problems. In the present study, blocking of the hydroxyl group of the salicylamide N-Mannich bases by O-acyloxymethylation has been shown to be a potentially useful approach to improve the stability. Various O-acyloxymethylated derivatives of N-(morpholinomethyl)salicylamide were prepared and found to be much more stable in acidic and neutral aqueous solution than the parent salicylamide N-Mannich base and to be readily converted to the latter in the presence of human plasma by enzymatic hydrolysis. In addition to providing an in vitro stabilizing effect the concept of O-acyloxymethylation makes it possible to obtain prodrug derivatives of a given amine drug with varying physicochemical properties of importance for drug delivery such as lipophilicity and water-solubility. This can simply be effected by the selection of an appropriate acyloxymethyl group.

Introduction

N-Mannich bases have been proposed as potentially useful prodrug candidates for NH-acidic compounds such as various amides, imides, carbamates, hydantoins and urea derivatives as well as for aliphatic or aromatic amines (Bundgaard, 1982; Bundgaard and Johansen, 1980a, b and c, 1981a, b and c; Johansen and Bundgaard, 1980a, b and c, 1981, 1982). Such N-Mannich bases were shown to decompose quantitatively to the parent compounds (for amide derivatives, see Scheme 1) in aqueous solution at rates being highly dependent on the pH of the solution and on various structural factors.

$$R\text{–}CONH\text{–}CH_2\text{–}NR_1R_2 + H_2O \rightarrow R\text{–}CONH_2 + CH_2O + R_1R_2NH$$

Scheme 1

In considering N-Mannich bases as prodrug forms for primary and secondary amines the amide-type component would act as a transport group. By N-Mannich base formation the pK_a of the amines is lowered by about 3 units (Bundgaard and Johansen, 1980a and b; Bundgaard, 1982). Therefore, by transforming amino compounds into N-Mannich base transport forms it would be pos-

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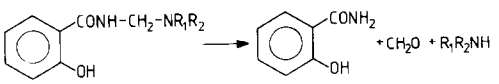
sible to increase the lipophilicity of the parent amines at physiological pH values by depressing their protonation, resulting in enhanced biomembrane-passage properties. This expectation of increased lipophilicity has been confirmed, for example, for the N-Mannich base derived from benzamide and ephedrine as well as phenylpropanolamine (Johansen and Bundgaard, 1982).

The selection of biologically acceptable amide-type transport groups affording an appropriate cleavage rate of an N-Mannich base of a given amine at pH 7.4 is, however, restricted. In a search for generally useful candidates it was observed (Johansen and Bundgaard, 1980b) that N-Mannich bases of salicylamides and different aliphatic amines including amino acids (Scheme 2) showed



Scheme 2

an unexpectedly high cleavage rate at neutral pH, thus suggesting the utility of salicylamide. As can be seen from Table 1 half-lives of decomposition of various salicylamide N-Mannich bases at pH 7.4 are of the same order of magnitude and considerably shorter than those of the corresponding benzamide N-Mannich bases (e.g. V).

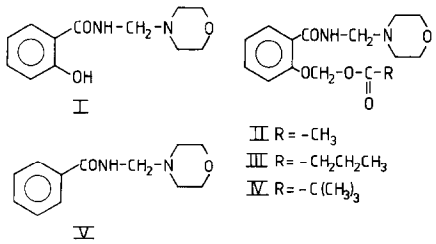
TABLE 1

HALF-LIVES OF DECOMPOSITION OF VARIOUS N-MANNICH BASES DERIVED FROM SALICYLAMIDE AND BENZAMIDE IN AQUEOUS SOLUTION AT 37°C

Compound	$t_{1/2}$ (min)	
	pH 2	pH 7.4
N-(Morpholinomethyl)salicylamide [a]	1.5×10^2	41
N-(Morpholinomethyl)benzamide [a]	1.2×10^3	1400
N-(Piperidinomethyl)salicylamide [a]	4.6×10^3	14
N-(Piperidinomethyl)benzamide [a]	1.7×10^4	47
N-(Methylaminomethyl)salicylamide [a]	–	28
N-(Methylaminomethyl)benzamide [a]	1.7×10^4	600
N-(α-Alanine-methyl)salicylamide [a]	1.2×10^2	17
N-(α-Alanine-methyl)benzamide [b]	3.3×10^2	190
N-(Sarcosin-methyl)salicylamide [b]	73	3
N-(Sarcosin-methyl)benzamide [b]	2.2×10^2	34

[a] From previous studies (Bundgaard and Johansen, 1980b; Johansen and Bundgaard, 1980b).
[b] Unpublished data.

Although the salicylamide N-Mannich bases are more stable in weakly acidic solutions (pH 2–5) than at pH 7.4 (Johansen and Bundgaard, 1980b) a drawback of this potential prodrug type requiring chemical (non-enzymatic) release of the parent amine drug is the limited in vitro stability, raising some stability-formulation problems. A possible means of improving the stability may be further derivatization of the salicylamide Mannich bases in such a manner that an enzymatic release mechanism is required prior to the spontaneous decomposition of the Mannich bases. Since the hydroxyl group in the salicylamide Mannich base is responsible for the great reactivity of these derivatives (cf. Table 1), possibly by intramolecular catalysis, blocking of this group may be expected to result in derivatives possessing a stability similar to that of benzamide Mannich bases. We have found that by performing such blocking through O-acyloxymethylation it is in fact possible to increase the stability in vitro and still obtain a rapid rate of amine release under conditions similar to those encountered in vivo due to the enzymatic lability of the O-acyloxymethyl group introduced. In the present paper the synthesis of a series of such O-acyloxymethylated derivatives (II–IV) of N-(morpholinomethyl)salicylamide (I) is reported along with results of a kinetic study of the chemical- and enzyme-mediated conversion of the compounds. Furthermore, determinations of the lipophilicity of the derivatives were performed.



Materials and Methods

Apparatus

Ultraviolet spectral measurements were performed with a Shimadzu UV-190 spectrophotome-

ter 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 PHM26 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. The chloromethyl esters of acetic and butyric acid were prepared as described by Ulich and Adams (1921).

Synthesis of salicylamide derivatives (I–IV)

N-(Morpholinomethyl)salicylamide (I). A mixture of 13.7 g of salicylamide, 8.7 g of morpholine, 8 ml of 37% aqueous formaldehyde and 130 ml of methanol was stirred at room temperature for 24 h. The solution was then evaporated in vacuo and the residue allowed to crystallize by standing overnight at 4°C. Recrystallization from chloroform–acetone afforded 8.2 g of the title compound, m.p. 126–127°C, reported m.p. 124–125°C (Gottstein et al., 1959) and 125–126°C (Singh and Agrawal, 1963).

O-(Acyloxymethyl)-N-(morpholinomethyl)salicylamide (II–IV). The compounds were synthesized by reaction of I with an iodomethyl ester prepared in situ by treatment of the corresponding chloromethyl ester with sodium iodide in acetone.

General procedure

O-(Acetoxymethyl)-N-(morpholinomethyl)salicylamide (II). Salt with one equivalent of fumaric acid. A mixture of I (1.19 g; 5 mmol) and potassium carbonate (1.39 g; 10 mmol) in acetone (30 ml) was stirred at room temperature for 30 min. In a separate flask chloromethyl acetate (0.72 g; 7.5 mmol) and dry potassium iodide (1.15 g; 7.5 mmol) in acetone (30 ml) were reacted at room temperature for 30 min. The iodomethyl acetate was transferred to the flask containing I and potassium carbonate by decantation. The mixture was refluxed for 4 h, filtered and the filtrate evaporated in vacuo. Water (10 ml) and ethyl acetate (35 ml) were added to the residue. The ethyl acetate phase was washed with 2 N sodium carbonate (10 ml) and twice with water (10 ml), dried, and evaporated. The residual oil was dissolved in ether (50 ml) and a solution of fumaric acid (0.46 g; 4 mmol) in 2-propanol (8 ml) was added followed by petroleum ether (10 ml). After standing at −20°C for 24 h the precipitate formed was collected and recrystallized from ethyl acetate. Yield: 0.90 g (42%), m.p. 109–111°C. ^{1}H NMR (DMSO-d_6) δ: 2.14 (s, 3H); 2.61 (t, 4H); 3.60 (t, 4H); 4.13 (distorted t, 2H); 5.89 (s, 2H), 6.58 (s, 2H), 7.0–7.7 (m, 4H), 8.3 (broad t, 1H).

Anal.: Calculated for $C_{19}H_{24}N_2O_9$: C, 53.77; H, 5.70; N, 6.60%. Found: C, 53.52; H, 5.79; N, 6.35%.

O-(Butyryloxymethyl)-N-(morpholinomethyl) salicylamide (III). Salt with 0.5 equivalent of fumaric acid. From I (1.19 g; 5 mmol) and chloromethyl butyrate (1.02 g; 7.5 mmol). Yield: 1.04 g (52%), m.p. 87–89°C (from ethyl acetate–petroleum ether). ^{1}H NMR (DMSO-d_6) δ: 0.86 (t, 3H); 1.58 (m, 2H); 2.36 (t, 2H); 2.55 (t, 4H); 3.62 (t, 4H); 4.18 (distorted t, 2H); 5.92 (s, 2H); 6.71 (s, 1H); 7.0–7.8 (m, 4H); 8.45 (broad t, 1H).

Anal.: Calculated for $C_{19}H_{26}N_2O_7$: C, 57.86; H, 6.65; N, 7.10%. Found: C, 57.61; H, 6.69; N, 7.08%.

O-(Pivaloyloxymethyl)-N-(morpholinomethyl) salicylamide (IV). Salt with 0.5 equivalent of fumaric acid. From I (2,38 g; 10 mmol) and chloromethyl pivalate (2.26 g; 15 mmol). Yield: 1.64 g (39%), m.p. 79–81°C (from ethyl acetate–petroleum

ether). The compound crystallized with 2/3 mole of water. ^{1}H NMR (DMSO-d_6)δ: 1.12 (s, 9H); 2.51 (t, 4H); 3.60 (t, 4H); 4.17 (d, 2H); 5.92 (s, 2H); 6.69 (s, 1H); 7.0–7.8 (m, 4H); 8.42 (broad t, 1H).

Anal: Calculated for $C_{20}H_{28}N_2O_7 \cdot 2/3\ H_2O$: C, 57.13; H. 7.03; N, 6.66%. Found: C, 57.20; H, 6.98; N, 6.51%.

O-(Acetoxymethyl)salicylamide (VI). The compound was prepared by reacting salicylamide with iodomethyl acetate as described above using column chromatography for purification, m.p. 92–93°C (from chloroform–petroleum ether). ^{1}H NMR ($CDCl_3$)δ: 2.05 (s, 3H); 5.88 (s, 2H); 7.0–7.8 (m, 3H); 8.1 (g, 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%.

Kinetic measurements

The hydrolysis of the salicylamide derivatives was studied in aqueous buffer solutions at 37.0 ± 0.2°C. Hydrochloric acid, acetate, phosphate and borate were used as buffers; 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 hydrolysis were followed by using a reversed-phase HPLC procedure. Mobile phase systems of 35–60% w/v methanol in 0.02 M phosphate buffer of pH 7.0 were used, the concentration of methanol being adjusted for each compound to give an appropriate retention time (3–10 min). The flow rate was 0.8 or 1.2 ml · min^{-1} and the column effluent was monitored at 230 nm (Spectra-Physics HPLC instrument) or 213 nm (Kontron HPLC 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 100–300 μl of a stock solution of the compounds in ethanol 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 hydrolysis were determined from the slopes of linear plots of the logarithm of residual salicylamide derivative against time.

The hydrolysis of the derivatives I–IV was also studied in human plasma diluted to 80% with 0.05 M phosphate buffer of pH 7.40 (at 37°C). At appropriate intervals 250 μl were withdrawn and added to 1000 μl of ethanol in order to deproteinize the plasma. After immediate mixing and centrifugation for 2–3 min, 10 μl of the clear supernatant was analyzed by HPLC as described above.

Determination of partition coefficients

The apparent partition coefficients of salicylamide and the derivatives I–IV were determined in octanol – 0.05 M phosphate buffer (pH 7.40) at 22°C. The aqueous phase and octanol were mutually saturated at 20–25°C before use. The compounds were dissolved in the aqueous phase and the octanol-buffer mixtures were shaken for 5–15 min to reach a distribution equilibrium. The volumes of each phase were chosen so that the solute concentration in the aqueous phase, before and after distribution, could readily be measured using the aforementioned HPLC methods. Centrifugation was used to separate the two phases. The partition coefficients (P) were calculated from Eqn. 1.

$$P = \frac{C_i - C_w}{C_w} \cdot \frac{V_w}{V_0} \quad (1)$$

where C_i and C_w represent the solute concentrations in the aqueous phase before and after distribution, respectively; V_w represents the volume of the aqueous and V_0 the volume of the octanol phase.

Results and Discussion

Hydrolysis of the derivatives I–IV in buffer solutions

The kinetics of breakdown of the derivatives II–IV was studied in aqueous solution at 37°C over the pH range 2–9.9. At constant pH and temperature the disappearance of the derivatives followed strict first-order kinetics over several half-lives as determined by HPLC (Fig. 1). The

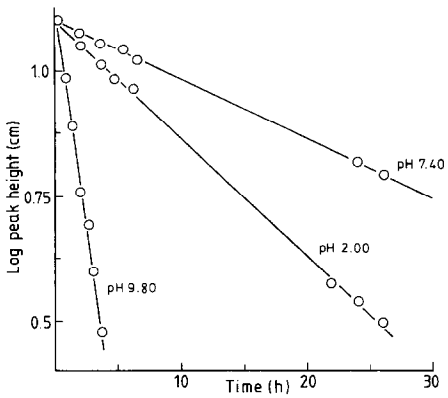


Fig. 1. First-order plots for the degradation of compound III in aqueous buffer solutions ($\mu = 0.5$) at 37°C.

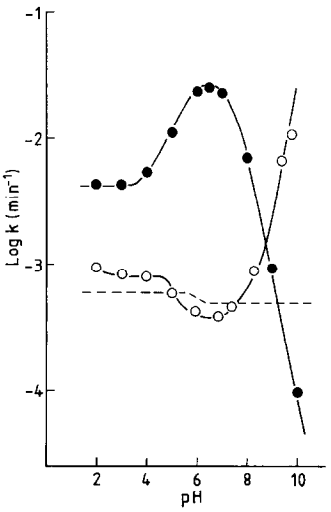


Fig. 2. The pH–rate profiles for the degradation of compound II (○), N-(morpholinomethyl)salicylamide (I) (●) and N-(morpholinomethyl)benzamide (V) (- - - - -) in aqueous solution at 37°C. The data for the latter two compounds were taken from previous studies (Bundgaard and Johansen, 1980b; Johansen and Bundgaard, 1980b).

pseudo-first-order rate constants (k) derived from such plots as shown in Fig. 1 are listed in Table 2. At the buffer concentration used (0.02 M) no significant general acid–base catalysis was observed.

Fig. 2 shows the pH–rate profile for the hydrolysis of compound II. For comparison the pH–rate profiles for the decomposition of the morpholino N-Mannich bases of salicylamide (I) and benzamide (V) obtained at similar conditions (37°C; $\mu = 0.5$) (Bundgaard and Johansen, 1980b; Johansen and Bundgaard, 1980b) are included in Fig. 2. As can be seen the overall stability of the O-acetoxymethyl derivative II is markedly greater than that of the parent N-Mannich base I at pH 2–8 and is very similar to that of N-(morpholinomethyl)benzamide (V) in this pH range.

TABLE 2

PSEUDO-FIRST-ORDER RATE CONSTANTS (k, in min^{-1}) FOR THE OVERALL DEGRADATION OF THE SALICYLAMIDE DERIVATIVES II–IV IN AQUEOUS SOLUTION ($\mu = 0.5$) AT 37°C

pH	Compound		
	II	III	IV
2.00	0.00094	0.0090	0.0010
3.00	0.00082		
4.00	0.00078		
4.98	0.00060		
6.00	0.00043		
6.88	0.00038		
7.40	0.00046	0.00046	0.00033
8.30	0.00088		
9.41	0.0065		
9.80	0.0106	0.0066	0.0013

The hydrolytic breakdown of the O-acetoxymethyl derivative II was examined in detail using an HPLC procedure capable of monitoring the disappearance of II and the time-course of hydrolysis products. In Fig. 3 is shown a typical chromatogram of a reaction solution of pH 7.4 at various reaction times. The disappearance of II was found to be accompanied by the formation of N-(morpholinomethyl)salicylamide (I) and O-(acetoxymethyl)salicylamide (VI), identified on the basis of their HPLC retention behaviour in comparison with those of authentic I and VI. Following their formation these products degraded into salicylamide. This final product of hydrolysis was formed in quantitative amounts ($100 \pm 3\%$) at all pH values studied as evidenced by HPLC analysis of completed reaction solutions. The time-courses of the various species are shown in Fig. 4 at various pH values and the proposed hydrolysis reactions taking place are depicted in Scheme 3, where k_1–k_4 are pseudo-first-order rate constants

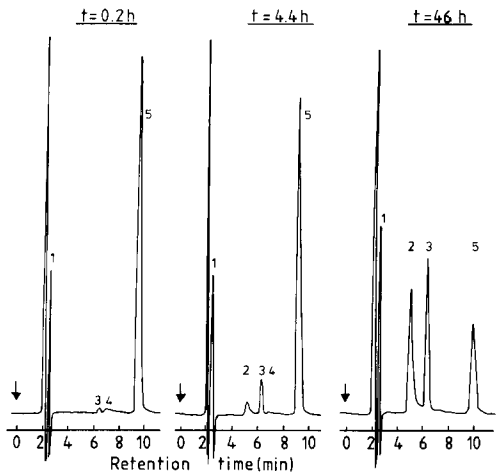


Fig. 3. High-performance liquid chromatographic traces of the hydrolysis of compound II (8×10^{-4} M) in 0.02 M phosphate buffer solution of pH 7.40 at 37°C. A 10-μl sample of the solution was chromatographed at the times indicated. Mobile phase used for HPLC: methanol–0.02 M phosphate pH 7.0 (2:3 v/v). Peak identities: 1, solvent front; 2, salicylamide; 3, compound VI; 4, compound I; 5, compound II.

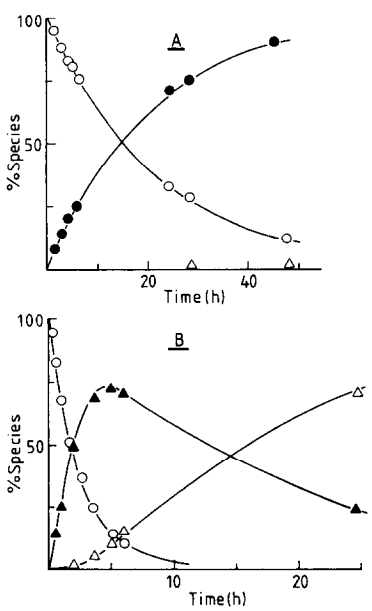
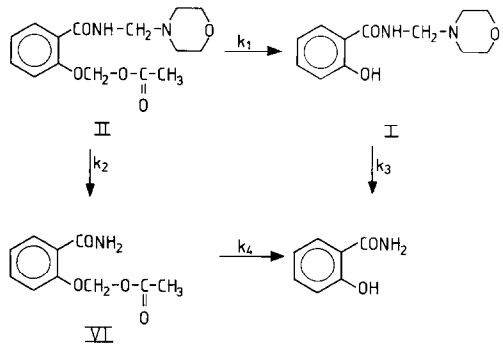


Fig. 4. Time-courses for compound II (○), compound VI (●), compound I (▲) and salicylamide (△) in the degradation of compound II at pH 3.00 (A) and pH 9.41 (B) (at 37°C). The concentrations at various times, expressed as percent of initial II concentration were determined by HPLC.



Scheme 3

for the depicted reactions. According to this scheme the pseudo-first-order rate constant for the overall degradation of compound II (k) can be expressed by:

$$k = k_1 + k_2 \qquad (2)$$

The relative importance of k_1 and k_2 depends on pH as revealed by the product analysis. In the pH range 2–7 the predominant degradation route of II is the k_2-reaction with hydrolysis of the N-Mannich base moiety. As seen in Fig. 4A compound VI is formed in almost 100% yield at pH 3. At pH 7.5–8 the k_1- and k_2-reactions make about the same contribution to the overall degradation of II whereas in alkaline solution the rate of hydrolysis of the ester moiety in II (k_1-reaction) greatly exceeds that of hydrolysis of the N-Mannich base moiety (cf. Fig. 4B). This stems from the fact that the ester is subject to hydroxide ion-catalyzed hydrolysis while the decomposition of the N-Mannich base moiety of II is independent of pH in alkaline solution, cf. the pH–rate profile for the related compound V (Fig. 2).

These observations based on the time-courses of the products I and VI were confirmed by comparing the pH–rate profiles for the hydrolysis of the products. As seen from Fig. 5 the O-acetoxymethyl salicylamide derivative VI is much more stable than compounds I and II at pH 2–8 whereas in alkaline solution the O-acyloxymethyl derivatives II and VI are less stable than compound I.

The shape of the pH-rate profile for compound

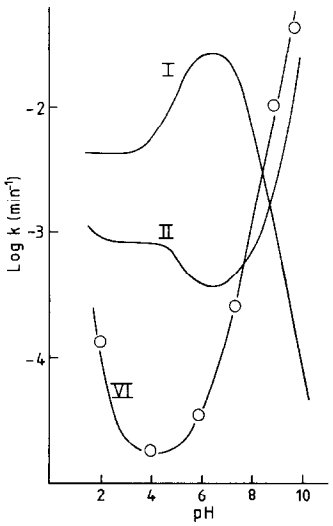


Fig. 5. The pH–rate profiles for the degradation of compounds I, II and VI in aqueous solution ($\mu = 0.05$) at 37°C.

II can be accounted for by the following rate expression:

$$k = \frac{k' \cdot K_a}{a_H + K_a} + \frac{k'' \cdot a_H}{a_H + K_a} + k_{OH} \cdot a_{OH} \qquad (3)$$

where K_a is the apparent ionization constant of the protonated N-Mannich base, a_H and a_{OH} are the hydrogen ion and hydroxide ion activity, respectively, k′ and k″ are the apparent first-order rate constants for the spontaneous decomposition of the unprotonated and protonated N-Mannich base species, respectively, and k_{OH} is a second-order rate constant for the specific base-catalyzed hydrolysis of the ester moiety of the compound. At pH less than 2 a specific acid-catalyzed reaction may also contribute to the overall degradation. The first two terms in Eqn. 3 are proposed on the basis of previous studies on the hydrolysis of benzamide N-Mannich bases (Bundgaard and Johansen, 1980a and b). The values of the rate constants derived from the pH–rate profile are listed in Table 3 along with the corresponding k′- and k″-values for N-(morpholinomethyl)benzamide. Inspection of Table 3 readily confirms that O-acyloxymethylation of a salicylamide N-Mannich base affords a derivative that behaves as the corresponding benzamide N-Mannich base in terms of stability in acidic and neutral aqueous solution.

As can be seen from Table 2 variation of the acyl residue in O-acyloxymethylated N-(morpholinomethyl)salicylamide does not result in any significant change in reactivity at pH 2 and 7.4, the rate of the overall hydrolysis at these pH values being determined by the rate of cleavage of the Mannich base moiety. In alkaline solution, on the other hand, the pivaloyl derivative IV is considerably more stable than the butyryl and acetyl derivatives. Under such pH conditions the ester moiety is the most sensitive portion, the rate of hydrolysis being a function of the polar and steric effects within the acyl groups. The variation of the

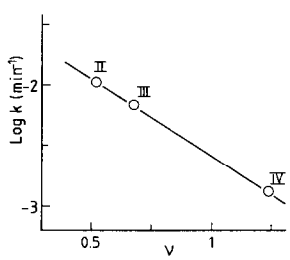


Fig. 6. Plot of log k (at pH 9.80; 37°C) vs the steric parameter (ν) for the O-acyloxymethyl salicylamide derivatives II–IV. The ν values (taken from Charton, 1977) refer to the alkyl moiety in the acyl groups, i.e. methyl, propyl and tertiary butyl.

TABLE 3

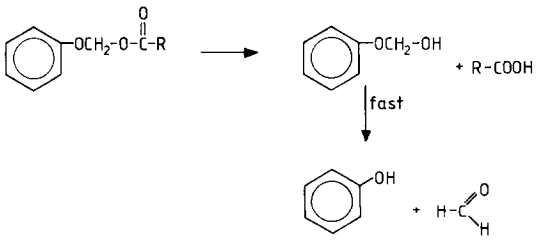
RATE CONSTANTS FOR THE DECOMPOSITION OF COMPOUND II AND N-(MORPHOLINOMETHYL)BENZAMIDE (V) IN AQUEOUS SOLUTION ($\mu = 0.5$) AT 37°C

Compound	k′ (min^{-1})	k″ (min^{-1})	k_{OH} ($M^{-1} \cdot min^{-1}$)	pK_a
Compound II	3.8×10^{-4}	8.2×10^{-4}	83.1	5.0
N-(Morpholinomethyl)benzamide [a]	5.0×10^{-4}	6.0×10^{-4}	–	5.55

[a] From Bundgaard and Johansen (1980b).

rates of hydrolysis of II–IV at pH 9.8 can fully be accounted for in terms of steric effects as seen from Fig. 6.

The hydrolysis of the acyloxymethyl ether moiety of compounds II–IV (and compound VI) is assumed to take place as shown in Scheme 4. The



Scheme 4

rate-determining step is hydrolysis of the ester grouping, resulting in the formation of a hemiacetal which spontaneously decomposes to phenol and formaldehyde (Fife and De, 1974; Loftsson and Bodor, 1982; Bodor et al., 1983).

Hydrolysis of compounds I–IV in plasma

The susceptibility of the derivatives II–IV to undergo a potential enzymatic hydrolysis was studied in vitro 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 and proceeded in all cases to give salicylamide in quantitative amounts through the intermediate formation of N-(morpholinomethyl)salicylamide (I). The degradation course observed for compound III in 80% plasma is shown in Fig. 7. Kinetic analysis, performed as described before (Buur et al., 1985), of the time-course for the intermediate N-(morpholinomethyl)salicylamide revealed a quantitative formation of this product, i.e. $k_1 \gg k_2$ (Scheme 3), $k \simeq k_1$. When all III had disappeared, i.e. after about 60 min, a plot of the logarithm of the percentage concentration of I against time produced a straight line from which a first-order rate constant of 0.011 min^{-1} was derived. When N-(morpholinomethyl)-salicylamide (I) was studied in 80% human plasma in a separate run, a first-order rate constant for its degradation of 0.010 min^{-1} was obtained.

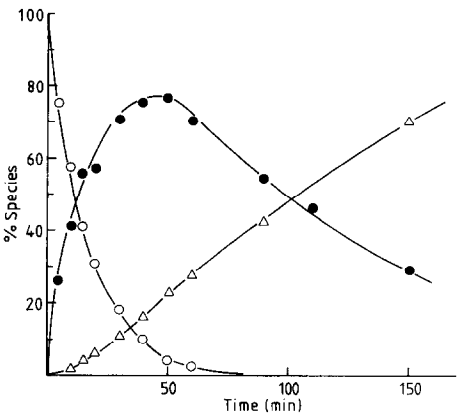


Fig. 7. Time-courses for compound III (○), compound I (●) and salicylamide (△) in the degradation of compound III in 80% human plasma solution (pH 7.4) at 37°C. The concentrations at various times, expressed as percent of the initial III concentration were determined by HPLC.

As appears from the observed rate data for the hydrolysis of the compounds I–IV (Table 4) plasma enzymes markedly accelerate the rate of hydrolysis, the butyryl derivative 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. Table 4 also shows that N-(morpholinomethyl)salicylamide is somewhat more stable in 80% plasma than in pure buffer solution. This decelerating effect by plasma was not observed in previous studies on various other N-Mannich bases but only 20% plasma solutions were used in these studies (Johansen and Bundgaard, 1981). Whether the observed effect is a special feature of compound I or would also be

TABLE 4

HALF-LIVES FOR THE HYDROLYSIS OF THE SALICYLAMIDE DERIVATIVES I–IV IN AQUEOUS BUFFER SOLUTION AND IN 80% HUMAN PLASMA AT 37°C

Compound	Half-lives	
	pH 7.4 buffer	80% plasma
I	41 min	66 min
II	25.0 h	77 min
III	25.5 h	12 min
IV	32.2 h	7.5 h

TABLE 5

PARTITION COEFFICIENTS (P) AND pK_a VALUES OF SALICYLAMIDE AND THE DERIVATIVES (I–IV)

Compound	log P [a]	pK_a
Salicylamide	1.15 [b]	8.05
I	1.27	5.25; 7.30 [c]
II	0.27	5.0
III	1.34	5.0
IV	1.81	5.0

[a] Partition coefficients between octanol and phosphate buffer of pH 7.4 at 22°C.

[b] Log P = 1.24 in octanol-water, reported log P 1.25 (Hansch and Leo, 1979).

[c] Macroscopic ionization constants (Johansen and Bundgaard, 1980b).

seen with other salicylamide N-Mannich bases remains to be examined.

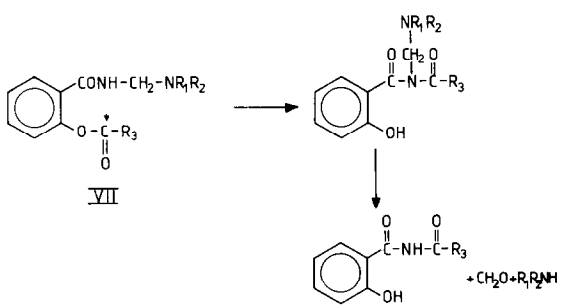
Lipophilicity of the O-acyloxymethyl derivatives

Partition coefficients (P) for the O-acyloxymethyl derivatives, compound I and salicylamide as determined using an octanol–aqueous buffer system (pH 7.4) are listed in Table 5. At pH 7.4 the derivatives II–IV are entirely present in the free base form (pK_a 5.0) whereas the phenolic group of compound I (pK_a 7.30) (Johansen and Bundgaard, 1980b) and salicylamide (pK_a 8.05) is partly ionized. The difference in the log P values for the derivatives II–IV is as expected on the basis of the π substituent values (Hansch and Leo, 1979).

Concluding Discussion

The results of the present study suggest that O-acyloxymethylation is a potentially useful approach to improve the suitability of salicylamide N-Mannich bases as prodrug forms for primary or secondary amino compounds. The O-acyloxymethylated derivatives of N-(morpholinomethyl)salicylamide are much more stable in acidic and neutral aqueous solution than the parent salicylamide N-Mannich base, and in the presence of plasma enzymes they are readily hydrolyzed to the latter. In addition to provide an in vitro stabilizing effect the concept of O-acyloxymethylation makes it possible to obtain prodrug derivatives of a given amine drug with varying physicochemical properties of primary importance for drug delivery such as water-solubility or lipophilicity. This is simply effected by the selection of an appropriate acyloxymethyl group as demonstrated above in case of lipophilicity.

Bioreversible blocking of the hydroxyl group in salicylamide N-Mannich bases may probably be performed by other means than the described O-acyloxymethylation. The most obvious means involving acylation to give simple ester derivatives (VII) is certainly not useful, however. Such compounds may not be expected to provide the desired stability in that they would be assumed to undergo a facile O → N acyl transfer reaction even in acidic solution (Scheme 5) in analogy with the behaviour of O-acylsalicylamides (Behme and Cordes, 1964; Gordon, 1967; Russell and Topping, 1975; Babhair and Hussain, 1983). The imide type N-Mannich bases arising from such intramolecular reaction should also be highly unstable due to the acidic nature of the imide (Bundgaard and Johansen, 1980a and b), the final products being N-acylsalicylamide, formaldehyde and amine (Scheme 5).



Scheme 5

Finally, it should be mentioned that the double prodrug concept described may also be useful as a means for developing prodrugs of salicylamide or drugs containing a salicylamide moiety. The phenolic group is known to be highly susceptible to undergo first-pass metabolic conjugation reactions (e.g. Barr and Riegelman, 1970; Shibasaki et al., 1981) and transient protection of this group via

O-acyloxymethylation may conceivably depress the metabolism and hence increase the bioavailability. Studies along this line with derivatives of the type II–IV and VI as well as other salicylamide derivatives are presently being undertaken in our laboratories.

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