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PRO-DRUGS AS DRUG DELIVERY SYSTEMS XIII. KINETICS OF DECOMPOSITION OF N-MANNICH BASES OF SALICYLAMIDE AND ASSESSMENT OF THEIR SUITABILITY AS POSSIBLE PRO-DRUGS FOR AMINES *

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SUMMARY

The kinetics of decomposition of various N-Mannich bases of salicylamide in aqueous solution at 37°C was studied to assess their suitability as pro-drugs for amino compounds. The decomposition, yielding salicylamide, amine and formaldehyde in stoichiometric amounts, showed bell-shaped pH–rate profiles which could be accounted for by assuming spontaneous decomposition of both neutral and protonated Mannich base and unreactivity of the derivatives in the anionic form. For the Mannich bases with the amines piperidine, α-alanine, methylamine and morpholine, the half-lives of decomposition at pH 7.40 and 37°C were 14, 17, 28 and 41 min, respectively, suggesting that salicylamide N-Mannich bases are possible candidates as pro-drugs for compounds containing a primary or secondary amino group. N-Amidomethylation of the amines with salicylamide resulted in a pronounced lowering of their basicity corresponding to 3–4 pK_a units which may be of potential utility for the application of N-Mannich bases as pro-drug forms for amines.

INTRODUCTION

Bioreversible derivatization of drug substances to produce pro-drugs with altered physicochemical properties can improve substantially both drug efficacy and safety as well as be a convenient approach in overcoming various pharmaceutical formulation problems such as lack of sufficient water solubility or dissolution rate of a given drug. In

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previous studies (Johansen and Bundgaard, 1979, 1980; Bundgaard and Johansen, 1980a, b and c), the concept of N-hydroxymethylation and N-aminomethylation of amides, imides, urea derivatives, hydantoins and various other NH-acidic compounds as a potentially useful means of obtaining pro-drug forms of such not easily derivatizable chemical entities were explored.

N-Mannich bases are formed by reacting an amide type compound with formaldehyde or, in rare cases, other aldehydes and a primary or secondary amine (N-aminomethylation or N-amidomethylation, respectively):

$$R - CONH_2 + CH_2O + R_1R_2NH \rightleftharpoons R{-}CONH{-}CH_2{-}NR_1R_2 + H_2O$$

In recent studies (Bundgaard and Johansen, 1980a and b), a great number of N-Mannich bases of various carboxamides, thioamides, sulphonamides, imides, urea derivatives and other NH-acidic compounds were shown to decompose quantitatively to the parent compounds in aqueous solution with rates highly dependent on pH and on structural factors including amide acidity, amine basicity and steric effects of the amine substituents. Besides cleavage rate large modifications in other physicochemical properties such as aqueous solubility, intrinsic dissolution rate and lipophilicity for the parent amide type compounds can be achieved by the appropriate selection of the amine component and by the proper choice of salt form (Johansen and Bundgaard, 1980).

In addition to be potential pro-drugs for amide type compounds N-Mannich bases may also be considered as pro-drug candidates for primary and secondary amines in which case the amide component would act as a transport group. The pK_a of amines decreases considerably by N-amidomethylation (about 3 pK_a units) (Bundgaard and Johansen, 1980b) and therefore, a potentially useful purpose for transforming amino compounds into N-Mannich base transport forms would be to increase the lipophilicity of the masked amines at physiological pH by depressing their protonation. A prerequisite of fulfilling this purpose is that the derivatives are converted to the parent amines within the body at a sufficiently high rate. However, the selection of biologically acceptable amide-type transport groups affording an appropriate cleavage rate of a Mannich base of a given amine at pH 7.4 is restricted. In a search for suitable and generally useful candidates we observed that N-Mannich bases of salicylamide and different amines exhibited an unexpectedly high cleavage rate at neutral pH, thus suggesting a potential utility of salicylamide. In the present paper the kinetics of decomposition of such Mannich bases are described along with data for the aqueous solubilities of the derivatives.

MATERIALS AND METHODS

Chemicals

The N-Mannich bases of salicylamide were prepared by reacting the amide with formaldehyde and amine according to previously described procedures: N-(morpholinomethyl)-salicylamide, m.p. 124–125°C (from ethyl acetate), rep. m.p. 124–125°C (Gottstein et al., 1959); N-(piperidinomethyl)salicylamide, m.p. 94–95°C (from methanol), rep. m.p. 93–95°C (Einhorn, 1905); N-(α-alaninomethyl)salicylamide, m.p. 164–165°C (from ethanol–water), rep. m.p. 159–161°C (Lauria et al., 1967). N-(methylaminomethyl)-salicylamide hydrochloride was prepared by the procedure described by Watase et al.

(1973), m.p. 141–143°C (from ethanol). The hydrochloride salt of N-(piperidinomethyl)salicylamide was prepared by treating the base with hydrogen chloride in methanol–ether, m.p. 152–154°C. Buffer substances and all other chemicals and solvents used were of reagent grade.

Apparatus

Ultraviolet and visible spectral measurements were performed with a Zeiss PMQ II spectrophotometer and a Perkin-Elmer 124 recording spectrophotometer, using 1-cm 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 uncorrected.

Kinetic measurements

All rate studies were performed in aqueous buffer solutions at 37.0 ± 0.2°C. Formate, acetate, phosphate, borate and carbonate were used as buffers; the total buffer concentration was 0.05 M except in experiments where buffer effects were studied specifically. A constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride.

Most reactions were monitored by measuring the amount of formaldehyde released during decomposition of the N-Mannich bases using a previously described modification (Johansen and Bundgaard, 1979) of the colorimetric method of Sawicki et al. (1961). The compounds were dissolved in the buffer solutions to give a concentration of about 8×10^{-4} M. The solutions were kept at 37°C and at appropriate intervals 1000-μl samples were withdrawn and diluted to 10.00 ml with water. A 500-μl sample of the dilution was then analyzed for formaldehyde as described previously (Johansen and Bundgaard, 1979). 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 625 nm) at infinity and at time t, respectively.

The decomposition of N-(piperidinomethyl)salicylamide was also followed by direct UV-spectrophotometry in the pH range 7–8. Reactions were performed in 2.5 ml aliquot portions of buffer solutions in a thermostatted quartz cuvette and were initiated by adding 25 μl of a stock solution of the compound in acetonitrile to give a final concentration of about 5×10^{-4} M. The reaction progress was followed by recording the decrease in absorbance at 330 nm as a function of time. Rate constants were determined from plots of $\log(A_t - A_\infty)$ against time.

Determination of ionization constants

The apparent macroscopic ionization constants of the N-Mannich bases were determined at 37°C by potentiometric titration of 10^{-3}–10^{-2} M aqueous solutions ($\mu = 0.5$) with standardized hydrochloric acid and/or sodium hydroxide solutions according to the procedure described by Albert and Serjeant (1971).

Solubility determinations

The solubility of salicylamide and some of its derivatives in water was determined at 22°C by an equilibrium procedure as previously described (Johansen and Bundgaard,

1980). The concentrations of the compounds in their saturated solutions were determined spectrophotometrically at 300 nm.

RESULTS AND DISCUSSION

Kinetics and mechanism of decomposition

The kinetics of breakdown of the N-aminomethylated salicylamide derivatives were studied in aqueous solution at 37°C over a wide range of pH. Under the experimental conditions used all reactions proceeded to completion as revealed by the formation of formaldehyde in stoichiometric amounts. At constant pH and temperature the reactions displayed strict first-order kinetics over more than 4 half-lives. In some runs the rates of breakdown of N-(piperidinomethyl)salicylamide were determined using both the colorimetric assay for formaldehyde and the direct UV-method. The values of the pseudo-first-order rate constants (k_{obs}) derived using these methods agreed within ±5%.

The rates of decomposition were found to be independent of buffer concentration from 0.02 to 0.1 M. Such lack of significant general acid–base catalysis was also noticed for the decomposition of N-Mannich bases of benzamide and various other carboxamides (Bundgaard and Johansen, 1980b).

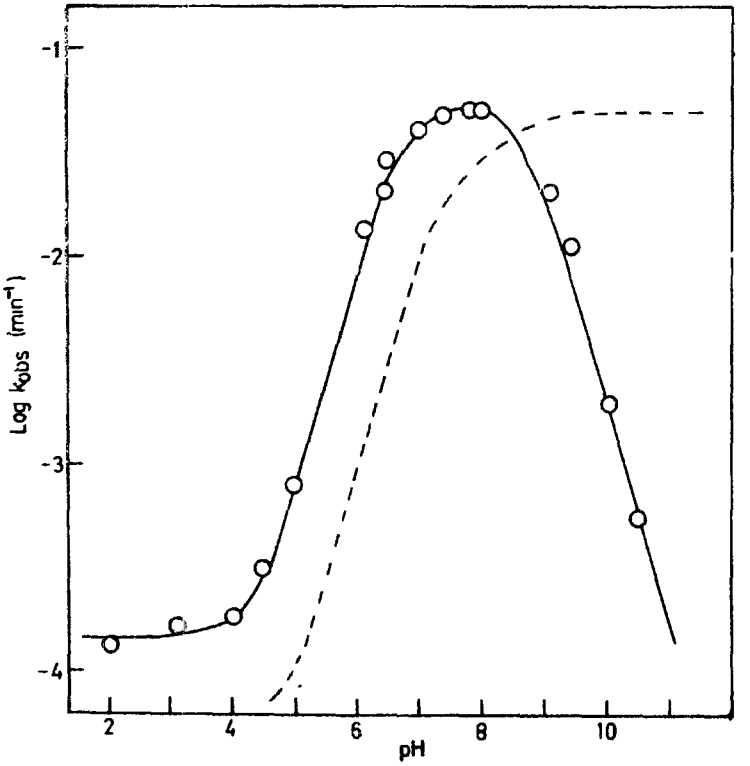


Fig. 1. The pH–rate profiles for the decomposition of N-(piperidinomethyl)salicylamide (———) and N-(piperidinomethyl)benzamide (– – –) in aqueous solution at 37° C.

The influence of pH on the degradation rate for some N-aminomethylated salicylamides is shown in Figs. 1 and 2, where the logarithms of the observed apparent first-order rate constants are plotted against pH. For the sake of comparison the pH–rate profiles for the corresponding N-Mannich bases of benzamide at the same experimental conditions (Bundgaard and Johansen, 1980b) are included in the figures. It is seen that whereas the benzamide derivatives show sigmoid pH–rate curves, the salicylamide N-Mannich bases exhibit bell-shaped curves. The shapes of the pH–rate profiles observed for the salicylamide N-Mannich bases with methylamine and α-alanine as the amine component were quite similar (at pH 5–10) to those for the piperidine and morpholine derivatives shown in Figs. 1 and 2.

The salicylamide N-Mannich bases can exist in 4 different forms. These are shown in Scheme 1, in which k_a, k_b, k_c and k_d are microscopic ionization constants, describing the interconversion of the 4 species denoted A, B, C and D. The macroscopic ionization constants, K_I, (for the protonated amino function) and K_{II} (for the phenol group) determined by titration are related to the microscopic ionization constants as shown in Eqns. 1–3:

$$K_I = k_a + k_b \quad (1)$$

$$1/K_{II} = 1/k_c + 1/k_d \quad (2)$$

$$K_I K_{II} = k_a k_c = k_b k_d \quad (3)$$

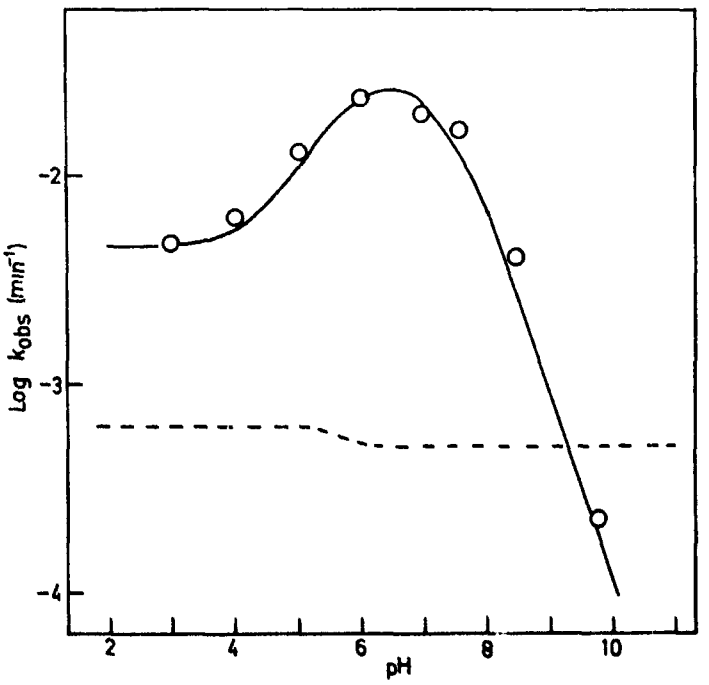
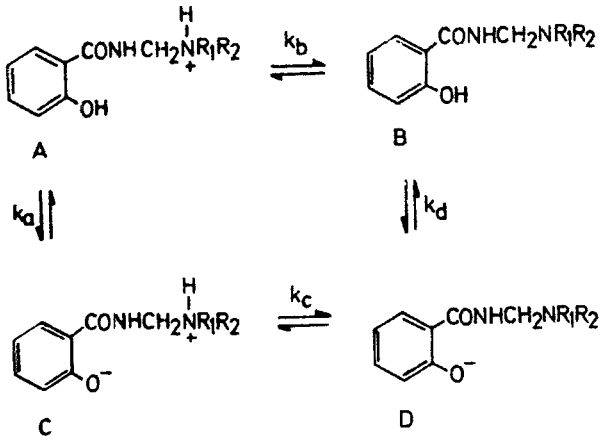


Fig. 2. The pH–rate profiles for the decomposition of N-(morpholinomethyl)salicylamide (——) and N-(morpholinomethyl)benzamide (– – –) in aqueous solution at 37°C.

SCHEME 1



The pH–rate profiles indicate that the anionic species (D) is unreactive and that the reactions in the pH-independent region below pH 3–4 are predominantly due to spontaneous decomposition of the protonated species (A). The bell-shaped curves can be accounted for in terms of spontaneous decomposition of the neutral (B) and/or the zwitterionic (C) forms. These species are related by a constant of proportionality which does not depend on pH:

$$\frac{[B]}{[C]} = \frac{k_c}{k_d} = \frac{k_b}{k_a} \tag{4}$$

Thus, the suggested reaction scheme is:

$$(B) \text{ and/or } (C) \xrightarrow{k_1} \text{products}$$

$$(A) \xrightarrow{k_2} \text{products} \tag{5}$$

and the equation for k_{obs} is accordingly:

$$k_{obs} = k_1(f_B + f_C) + k_2 f_A \tag{6}$$

where f is the fraction of the N-Mannich base present as the specified ionic form at the pH of the measurement, and k_1 and k_2 are apparent first-order rate constants for the spontaneous degradation of (B) and/or (C) respectively (A). The rate constant k_2 was calculated from the rate data in the pH-independent region and k_1 from the remaining data along with the following expression for the fractions of (B) and (C):

$$(f_B + f_C) = \frac{K_I a_H}{a_H^2 + K_I a_H + K_I K_{II}} \tag{7}$$

where a_H is the hydrogen ion activity as determined by a glass electrode. Eqn. 7 was

derived from Eqns. 1–3 and the equations defining the microscopic ionization constants (e.g. $k_a = a_H[C]/[A]$).

The derived rate constants and macroscopic ionization constants for the salicylamide N-Mannich bases with piperidine, morpholine and methylamine are listed in Table 1. The solid curves in Figs. 1 and 2 were constructed from Eqn. 6 and the good agreement observed between the calculated and experimental data demonstrates that the reaction scheme of Eqn. 5 adequately describes the degradation kinetics.

The proposed kinetic scheme for the reactions of N-Mannich bases of salicylamide is similar to that previously shown to account for the decompositions of N-Mannich bases of benzamide (Bundgaard and Johansen, 1980a and b). These reactions were analyzed in terms of spontaneous decomposition of the free-base form (k_1) and of the conjugate acid form (k_2). The mechanism proposed for the k_1-reaction (which predominates in weakly acidic to basic solution) involves as rate-determining step a unimolecular N–C bond cleavage with formation of an amide anion and an immonium cation. In subsequent fast steps, a solvent molecule transfers a proton to the amide anion and a hydroxide ion to the immonium ion, giving methylolamine, which rapidly dissociates to formaldehyde and amine (Bundgaard and Johansen, 1980b) (Scheme 2). The unusual pH–rate profiles for the salicylamide derivatives in comparison with those for the benzamide derivatives arise from the greatly decreased reactivity of the derivatives in the form where the phenolic hydroxy group is ionized. This stabilizing effect of the phenolate anion may be accounted for on basis of the proposed degradation mechanism since the electron displacement depicted in Scheme 2 and leading to N–C bond cleavage should be depressed by the ortho-positioned phenolate anion. Since the anionic species (D) is unreactive the zwitterionic form (C) may also make no contribution to the reactions so the k_1-reaction in Eqn. 5 is likely to be due solely to reaction of the neutral form (B).

Comparing the rate data for salicylamide and benzamide Mannich bases with the same amine components (Table 1) shows that the salicylamide derivatives are more reactive with respect to both the k_1-reaction and the k_2-reaction. As a consequence a greater reactivity is manifested in acidic to slightly alkaline solutions (cf. Figs. 1 and 2). The struc-

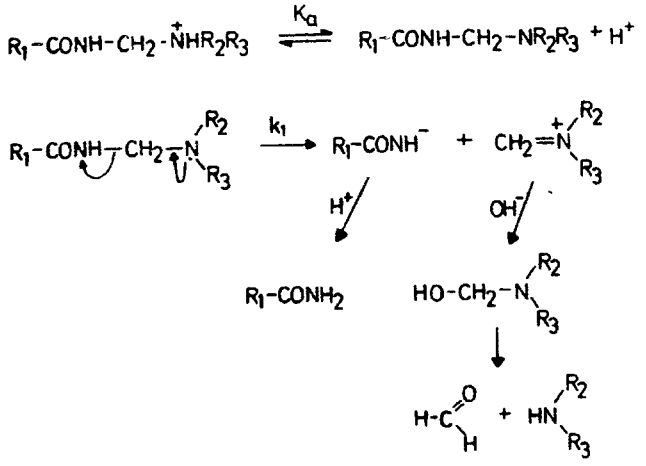
TABLE 1

IONIZATION CONSTANTS AND RATE CONSTANTS FOR THE DECOMPOSITION OF VARIOUS N-MANNICH BASES IN AQUEOUS SOLUTION (μ = 0.5) AT 37°C *

Compound	k_1 (min^{-1})	k_2 (min^{-1})	pK_a^I	pK_a^{II}
N-(Piperidinomethyl)salicylamide	0.074	1.5×10^{-4}	6.95	8.45
N-(Morpholinomethyl)salicylamide	0.027	4.5×10^{-3}	5.25	7.30
N-(Methylaminomethyl)salicylamide	0.027	n.d.	6.30	8.95
N-(Piperidinomethyl)benzamide	0.051	4.0×10^{-5}	7.75	
N-(Morpholinomethyl)benzamide	0.0005	6.0×10^{-4}	5.55	
N-(Methylaminomethyl)benzamide	0.0026	4.0×10^{-5}	7.50	

* The data for the benzamide derivatives are from a previous study (Bundgaard and Johansen, 1980b).

SCHEME 2



tural effects on the decomposition rate of N-Mannich bases have previously been shown to involve steric effects and basicity of the amine component and acidity of the amide component (Bundgaard and Johansen, 1980b). The rates of both the k_1- and k_2-reactions were found to increase with decreasing pK_a of the Mannich base and the greater reactivity of the salicylamide derivatives may at least in part be a reflection of their lower pK_a values (i.e. pK_a^I referring to the amino function), cf. Table 1. It is noteworthy that the rate enhancement of the k_1-reactions on going from benzamide to salicylamide derivatives differs greatly for the 3 amines studied. For the morpholine compound the rate enhancement corresponds to a factor of 54 while the methylamine and piperidine derivatives show rate enhancements of 10- and 1.5-fold. The magnitude of the rate enhancement may possibly be related to the basicity of the amines in that it apparently parallels the order of decreasing basicity of the amines: the pK_a for the amines is 11.12 (piperidine), 10.66 (methylamine) and 8.33 (morpholine) at 20°C (Albert and Serjeant, 1971).

The solubility of the N-Mannich bases

The water solubility of the derivatives was measured and compared to that of salicylamide. The following results were obtained (at 22°C): salicylamide, 0.019 mol/l; N-(methylaminomethyl)salicylamide hydrochloride, 0.040 mol/l; N-(piperidinomethyl)-salicylamide hydrochloride, 4.2 mol/l. As can be seen, greatly increased solubility is observed for the piperidine derivative in contrast to the methylamine compound. Such difference was also observed for the corresponding benzamide Mannich bases and it was attributed to the occurrence of intramolecular hydrogen bonding in the Mannich bases prepared from primary amines (Johansen and Bundgaard, 1980).

N-Mannich bases of salicylamide as pro-drug candidates for amines

The results of the present study suggest that N-Mannich bases of salicylamide may be of potential usefulness as pro-drugs of primary and secondary amino compounds. The derivatives undergo a quantitative conversion to the parent compounds in aqueous solution with rate maxima occurring in the range, pH 6–8. At pH 7.40 and 37°C the half-lives of decomposition were found to be 14, 17, 28 and 41 min for the piperidine, α-ala-

nine, methylamine and morpholine derivatives, respectively. These figures are of the same order of magnitude which contrast greatly with the widely different reactivities of benzamide Mannich bases of the same amines, e.g. $t_{1/2}$ for the morpholine and piperidine derivatives are 1400 and 47 min, respectively, at pH 7.40 and 37°C (Bundgaard and Johansen, 1980a and b). Thus it appears that salicylamide may be a generally applicable transport group for amines in the form of N-Mannich bases. As noted in the introduction the basicity of amines decreases markedly by N-amidomethylation. In the case of salicylamide, comparison of the pK_a^I values in Table 1 with the pK_a values for the amines quoted above shows that N-amidomethylation of the amines with salicylamide results in a lowering of pK_a of 3–4 units.

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