Kinetic Study on Hydrolysis of a Mannich Base Compound

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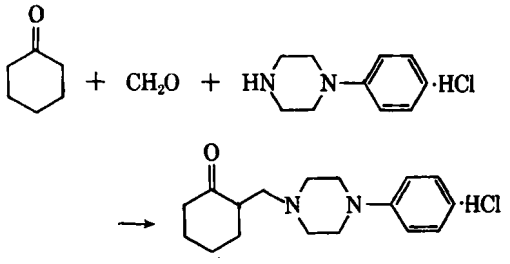
The hydrolysis of 2-(4-phenyl-1-piperazinylmethyl)cyclohexanone hydrochloride, a Mannich condensation product, was studied in buffered aqueous solutions in the pH range of 1.1 to 5.5. The hydrolytic products, *N*-phenylpiperazine and a rearranged dimer of 2-methylenecyclohexanone, were isolated and characterized. The hydrolysis was specific acid and general base catalyzed with maximum stability around pH 2. The hydrolysis was pseudo first order in nature with an energy of activation, uncorrected for the heat of ionization of water, of 27.3 Kcal./mole.

THE MANNICH condensation reaction is frequently used in the synthesis of pharmaceutical compounds. In 1922, Mannich and Heilner (1) observed that ω-dimethylaminopropiophenone hydrochloride prepared from acetophenone, formaldehyde, and dimethylamine hydrochloride decomposed on steam distillation to give acrylophenone and dimethylamine hydrochloride. Similar results were noted by Blicke and Burckhalter (2). Mannich (3) and Mannich and Hönig (4) also had reported that 2-(piperidinomethyl)cyclohexanone on fusion decomposed to piperidine hydrochloride and 2-methylenecyclohexanone.

References on the breakdown of Mannich bases have been essentially qualitative. Stability studies in this laboratory on a pharmaceutical product formed by a Mannich base reaction showed an unexpected hydrolysis and afforded an opportunity to investigate quantitatively the kinetics of the hydrolysis of this compound. Factors affecting hydrolysis which could be utilized as guidelines for pharmaceutical formulation were of particular interest.

2-(4-Phenyl-1-piperazinylmethyl)cyclohexanone hydrochloride (compound MA1050) was prepared by the Miles Chemical Therapeutics Research Laboratory by the condensation of cyclohexanone, formaldehyde, and *N*-phenylpiperazine hydrochloride as shown in Scheme I.



Scheme I

This compound was stable to moderate amounts of dry heat, but broke down under accelerated conditions of combined heat and humidity. In accordance with findings on similar compounds (3, 4), the methylene-nitrogen bond was considered the most probable point of hydrolytic attack. This was confirmed by the identification of the breakdown products. Analytical procedures were adapted to follow the appearance of one of the degradation components, which introduced into the system a new functional group, a secondary amine.

EXPERIMENTAL

Isolation and Characterization of Degradation Compounds.—Approximately 2.0 Gm. of compound MA1050 was dissolved in about 100 ml. of 1.0 *N*-hydrochloric acid and digested on a steam bath for a few hours. The solution was cooled and extracted with several portions of ethyl ether. The ether extract was discarded. The aqueous phase was made alkaline with sodium hydroxide solution and extracted with ethyl ether. The ether extract was washed free of alkali, dried with anhydrous sodium sulfate, and the ether evaporated. The residue was identified as *N*-phenylpiperazine by its infrared spectrum and by the melting point of its hydrochloride.

A second nonnitrogenous product was isolated by heating a 6% aqueous solution of MA1050 in a closed system for a few hours on a steam bath. A white crystalline material separated. This was recrystallized from aqueous ethanol and dried *in vacuo* at 60° for 3 hours, m.p. 156–159°. This material was characterized as the hydrated dimer of 2-methylenecyclohexanone which was previously reported by Warnhoff and Johnson (5).[1] The melting point checked more closely with that reported by Mannich (3) for the same compound.

Analytical Method for Determining the Rate of Hydrolysis.—The rate of hydrolysis was followed by determining the rate of appearance of *N*-phenylpiperazine. A color reaction of secondary amines with carbon disulfide and cupric ion reported by Umbreit (6) was adapted for this purpose. The copper dithiocarbamate of the amine formed in the reaction was extracted into benzene and determined colorimetrically. This reaction was suitable for

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[1] The authors thank Dr. O. E. Fancher, Director of the Chemical Therapeutics Research Laboratory, Miles Laboratories, Inc., for characterizing this degradation product.

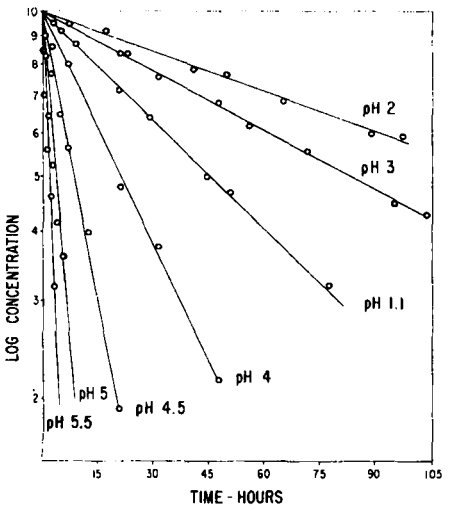


Fig. 1.—First-order plot of the hydrolysis of compound MA1050 in buffered aqueous solutions at 60°.

TABLE I.—OBSERVED FIRST-ORDER SPECIFIC REACTION CONSTANTS (k IN HOUR^{-1}) FOR THE HYDROLYSIS OF COMPOUND MA1050 IN BUFFERED AQUEOUS SOLUTIONS AT 60°[a]

pH	k, hr.$^{-1}$ × 10^3
1.1	15.30
2.0	5.63
3.0	8.46
4.0	31.72
4.5	78.70
5.0	205.70
5.5	382.70

[a] The ionic strength of all solutions was 0.15.

determining *N*-phenylpiperazine with no interference from compound MA1050 or its nonnitrogenous breakdown product. *N*-Phenylpiperazine was stable under the experimental conditions of hydrolysis.

Procedure.—The following general procedure was used for studying the rate of hydrolysis. Samples of 0.2 Gm. of compound MA1050 were weighed accurately into 200-ml. volumetric flasks containing an amount of potassium chloride to adjust the ionic strength to a predetermined value. The contents were dissolved in the appropriate buffer solution preheated to the particular temperature at which the hydrolysis was studied. More of the hot buffer solution was added to volume, the contents mixed, and the flask placed in a constant temperature bath. Fifteen-milliliter aliquots were withdrawn after 5 minutes equilibration and thereafter at periodic intervals. These solutions were cooled to room temperature, the pH adjusted to 6 with 0.2 *N* NaOH, transferred to a 100-ml. volumetric flask, and water added to volume. A 2.0-ml. aliquot of each was assayed for *N*-phenylpiperazine. The comparative color developed simultaneously on a 2.0-ml. aliquot of a suitable standard solution of *N*-phenylpiperazine hydrochloride was used in calculating the concentration of the sample.

pH and Temperature Effect.—The effect of pH and temperature on the rate was determined by the hydrolysis at three different temperatures in buffer solutions as follows: pH 1.1, 2, 3, 4, 4.5, 5, and 5.5 at 60°, and pH 3, 4, and 5 at 50° and 70°. Hydrochloric acid was used for pH 1.1 solution; phosphoric acid and monobasic sodium phosphate were used for solutions of pH 2 and 3, and acetic acid and sodium acetate for pH 4, 4.5, 5, and 5.5. The ionic strength of all solutions was adjusted to 0.15 with potassium chloride. Buffers of pH higher than 5.5 could not be employed in this study due to precipitation of free base of the compound.

Effect of Initial Concentration on the Order and Rate of Hydrolysis.—The procedure, as outlined, was used for studies in acetate buffer at pH 5 at 60° using two different initial concentrations of compound MA1050.

Effect of Ionic Strength.—The effect of ionic strength on the rate was investigated by the hydrolysis of compound MA1050 at 60° in solutions of pH 1.1, 2, 3, 4, and 5. The ionic strengths were adjusted to different levels with potassium chloride.

Buffers as General Acid or General Base Catalysts.—The effect of buffer constituents as general acid or general base catalysts was investigated by hydrolyzing compound MA1050 in solutions of pH 2, 3, 4, and 5 at constant ionic strength but different concentrations of the buffer species at each pH.

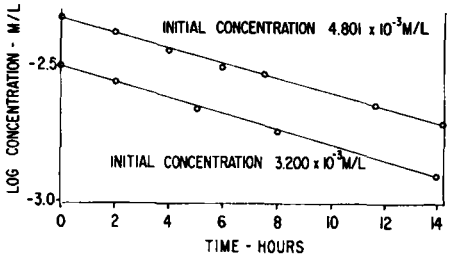


Fig. 2.—Plot showing the effect of initial concentration on the rate of hydrolysis of Compound MA 1050 in acetate buffer pH 5 at 50°.

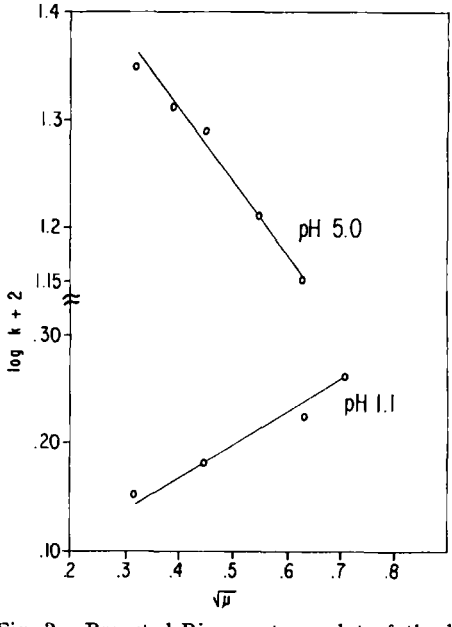


Fig. 3.—Bronsted-Bjerrum-type plot of the logarithm of the observed rate constants against $\sqrt{\mu}$ for the hydrolysis of compound MA1050 at pH 1.1 and 5 at 60°.

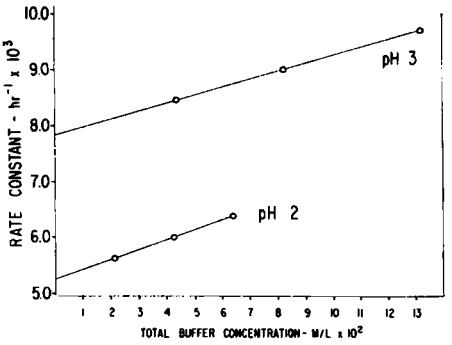


Fig. 4.—Effect of buffer concentration on the hydrolysis of Compound MA1050 in phosphate buffers of pH 2 and 3 at 60° at constant ionic strength.

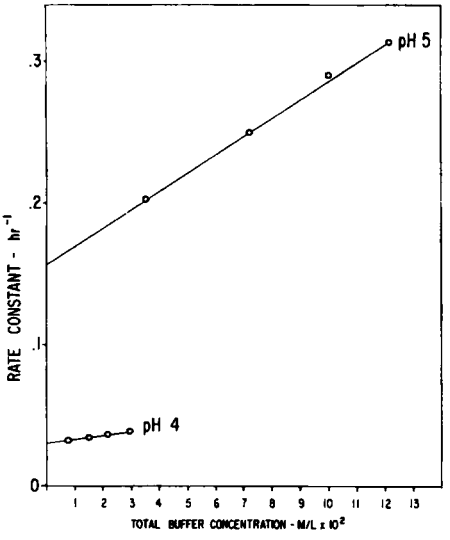


Fig. 5.—Effect of buffer concentration on the hydrolysis of compound MA1050 in acetate buffers of pH 4 and 5 at 60° at constant ionic strength.

RESULTS AND DISCUSSION

Figure 1 is a typical plot of the logarithm of the concentration remaining in solution against time for the hydrolysis of compound MA1050 and demonstrates that the hydrolysis is pseudo first order with respect to compound MA1050 and is a function of the pH of the medium. The first-order rate constants for the reaction at 60° are given in Table I.

Figure 2 demonstrates that the rate is directly dependent on the concentration of MA1050.

Effect of Ionic Strength.—No salt was noticed at pH 2. At pH 3, 4, and 5, an increase in the ionic strength resulted in a decrease in rate. At pH 1.1, however, increasing the ionic strength had the reverse effect of accelerating the rate. The data were tested using the Bronsted-Bjerrum equation (Eq. 1) for bimolecular reactions.

$$\log k = \log k_0 + 2QZ_AZ_B\mu^{1/2} \quad \text{(Eq. 1)}$$

where k is the observed rate constant, k_0 is the rate constant at zero ionic strength, Q is a function of such properties of the solution as dielectric constant, temperature, etc., and thermodynamic constants; Z_A and Z_B are the charges on the reacting species, and μ is the ionic strength of the medium.

Figure 3 is a plot of the logarithm of the observed rate constants against the square root of the ionic strengths for the hydrolysis at pH 1.1 and 5 at 60°. The positive slope for the hydrolysis at pH 1.1 indicates a reaction between ions of like charges. Conversely, the negative slope for the hydrolysis at pH 5 indicates a reaction between ions of unlike charges. At pH 2, where there is no salt effect, the value of Z_AZ_B is zero, which would be consistent for a reaction between uncharged species or between an ion and a neutral molecule. The values for Z_AZ_B were determined from the slopes of the lines in Fig. 3, and from the calculated values for Q at 60°. These were 0.28 and −0.58 at pH 1.1 and 5, respectively. The finding of nonintegral values for Z_AZ_B could be due to high ionic strength, the occurrence of more than one reaction at the point of determination, or to a significant contribution by the secondary salt effect.

Effect of Buffers as General Acid or General Base Catalysts.—Under the experimental conditions used in this study, compound MA1050 was subject to specific acid and general base catalysis. The observed rate constant is a summation of the catalytic effect of all the species involved. In accordance with the treatment of Frost and Pearson (7) it can be represented as

$$k_{obs} = k_1(H^+) + k_2(OH^-) + \Sigma_i k_i(HA_i) + \Sigma k_i(A^-_i) + \Sigma k_i(B_i) + \Sigma k_i(B_iH^+) \quad \text{(Eq. 2)}$$

where HA is a general acid and A^- its conjugate base, and B is a general base and BH^+ its conjugate acid. Figures 4 and 5 are plots of the observed rate constants against the total buffer concentrations at pH 2, 3, 4, and 5 at 60°, the ionic strength of all solutions being the same. No attempt was made to calculate separately the specific contribution of the individual general acids or general bases to the total buffer catalysis. The increasingly positive slopes of the lines with increasing pH indicate that the catalytic effect of the various species was limited to their basic forms.

pH-Rate Profile.—According to Eq. 2, the plots of the observed rate constants against buffer concentrations extrapolated to zero buffer concentration give the rate constant uninfluenced by the buffer species. These values were obtained from Figs. 4 and 5 and plotted as a function of pH in Fig. 6 to show the pH dependency of the rate and a maximum stability at pH 2.

A number of chemical reactions can lead to the same observed experimental dependence. Representing the basic form of compound MA1050 as A and a general base as B, the probable reactions are

$$1,\ A^{++} + H^+ \xrightarrow{k_{H^+}}$$

$$2,\ A^{++} + H_2O \xrightarrow{k_{H_2O}}$$

$$3,\ A^{++} + B \xrightarrow{k_B}$$

$$4,\ A^+ + H^+ \xrightarrow{k'_{H^+}}$$

$$5,\ A^+ + H_2O \xrightarrow{k'_{H_2O}}$$

$$6,\ A^+ + B \xrightarrow{k'_B}$$

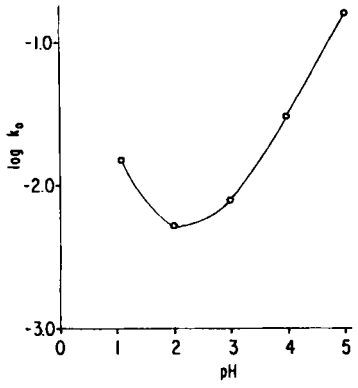


Fig. 6.—Effect of pH on the hydrolysis of compound MA1050 in buffered aqueous solutions at 60° and at constant ionic strength.

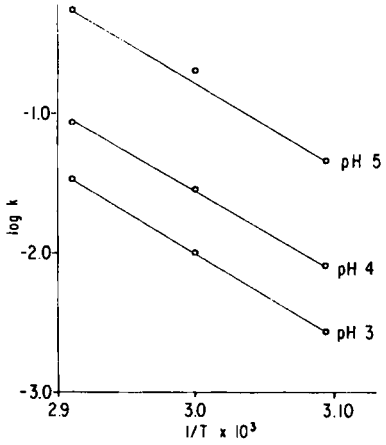
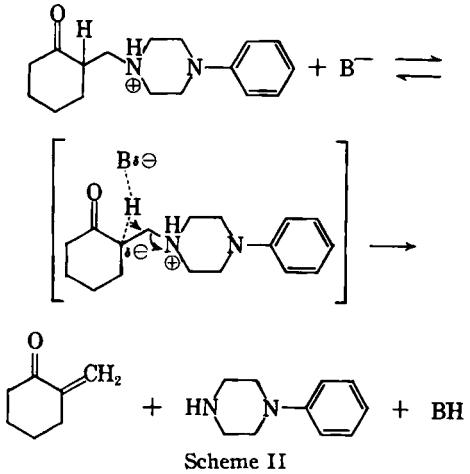


Fig. 7.—Arrhenius-type plot of the logarithm of the observed rate constants against the reciprocal of absolute temperature for the hydrolysis at pH 3, 4, and 5.

Reaction *3* can be ruled out because one of the nitrogens in compound MA1050 is weakly basic and under conditions where it is protonated the hydroxyl ion concentration will be negligible. The predominant reactions appear to be *1*, occurring below pH 2, and *6*, occurring at higher pH. Theoretically, the slopes of the plot in Fig. 6 should be an indication of the order of the reaction with respect to the hydrogen and hydroxyl ion concentrations. The numerical value of the slope varies from 0 to 0.72. These fractional values are an indication of the complexity of the reaction in the pH region under study.

Temperature Effect on the Rate.—The effect of temperature on the rate constants is demonstrated by the Arrhenius plot (Fig. 7). Data obtained at pH 3, 4, and 5 at 50°, 60°, and 70° were used in this plot. The energy of activation, uncorrected for the heat of ionization of water, was calculated from the slopes of the lines and presented in Table II. The average value for the energy of activation was 27.3 Kcal./mole. The frequency factor, A, and the entropy of activation, ΔS^{+}, were calculated from this value using standard equations. These values are given in Table II. The frequency factor was in the range of 10^{12} to 10^{13}, which seems high for a bimolecular reaction. Of interest was the entropy of activation change from a negative value at pH 3 to a positive value at pH 5, for which no rationale could be developed.

Mechanism of Hydrolysis.—The isolation and characterization of the degradation products of compound MA1050 as the hydrated, rearranged dimer of 2-methylenecyclohexanone and *N*-phenylpiperazine indicate that the primary path of breakdown is the hydrolytic attack at the methylene-nitrogen bond. The hydrolysis is first order with respect to the concentration of compound MA1050, but of a complex order with respect to hydrogen and hydroxyl ion concentrations making if difficult to write a rate equation. There is a positive salt effect at pH 1.1, no salt effect at pH 2, and a negative salt effect at higher pH, which suggests that more than one mechanism is involved. Although this study was not designed to investigate the mechanism of hydrolysis of compound MA1050, two pathways can be postulated, which appear to be in agreement with the kinetic data. In solutions of pH higher than 2, a pathway consistent with the negative salt effect and the marked catalytic effect of the acetate ion at pH 5 would be as shown in Scheme II.

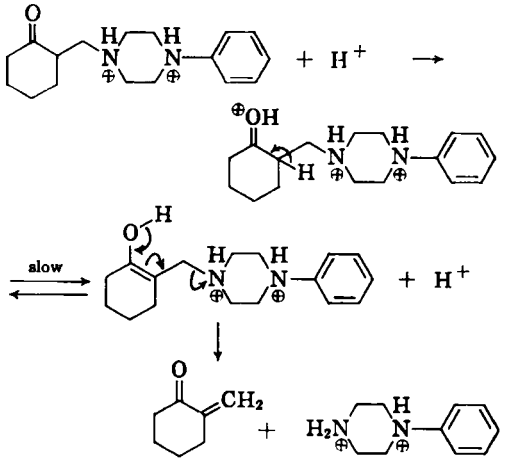


Scheme II

In solutions of pH lower than 2, the pathway might be as shown in Scheme III.

TABLE II.—VALUES FOR THE ENERGY OF ACTIVATION, FREQUENCY FACTOR, AND THE ENTROPY OF ACTIVATION CALCULATED FROM THE SLOPES OF THE ARRHENIUS PLOT

	pH 3			pH 4			pH 5		
	50°	60°	70°	50°	60°	70°	50°	60°	70°
Frequency factor, A, sec.$^{-1}$	2.1×10^{12}	1.9×10^{12}	6.0×10^{11}	6.5×10^{12}	7.1×10^{12}	6.0×10^{12}	3.7×10^{13}	1.9×10^{13}	3.9×10^{13}
Entropy of activation, cal./mole deg.	−2.28	−2.57	−4.93	−0.08	0.04	0.35	3.36	2.00	3.34
Energy of activation, Ea, Kcal./mole	27.71			26.85			27.31		



Scheme III

The 2-methylenecyclohexanone shown in the postulated reactions appears to dimerize and rearrange according to the pathway suggested by Warnhoff and Johnson (5).

Pharmaceutical Consideration.—The immediate practical application of this study was in the elimination or control of factors affecting the rate of breakdown of a parenteral preparation for clinical pharmacological work. The pH of an aqueous solution of compound MA1050 is 5.0. The kinetic data indicated that a solution at this pH could not be sterilized by autoclaving, nor could a sterile solution be prepared aseptically and stored even at refrigeration temperature for reasonable lengths of time without appreciable breakdown. Formulation considerations were therefore directed to the prepasation of a sterile lyophilized powder.

SUMMARY

A Mannich base, 2-(4-phenyl-1-piperazinylmethyl) cyclohexanone, was susceptible to hydrolytic attack at its methylene-nitrogen linkage. The breakdown products were isolated and characterized as *N*-phenylpiperazine and the hydrated dimer of 2-methylenecyclohexanone.

A kinetic study of the hydrolysis reaction in buffer solutions of pH 1.1 to 5.5 and factors influencing it are reported. The hydrolysis was pseudo first order in nature and was specific acid and general base catalyzed. The pH profile showed the compound to have maximum stability in solution at pH 2. At pH values other than 2, ionic concentration of the medium affected the rate. The energy of activation for the hydrolysis in buffered aqueous solutions, uncorrected for the heat of ionization of water, was 27.3 Kcal./mole. In view of the uncertainty of the hydrogen or hydroxyl ion dependency, no rate equation is given. However, two probable pathways for the hydrolysis are suggested.

Knowledge from the evaluation of stability factors influencing compound MA1050 was applied to pharmaceutical considerations with the development of a lyophilized product as the most stable dosage form.

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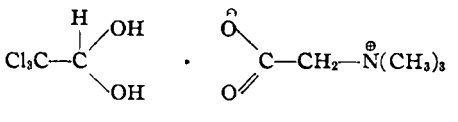
Drug Standards

Qualitative and Quantitative Tests for Chloral Betaine

Provisional, unofficial monographs are developed by the Drug Standards Laboratory, in cooperation with the manufacturers of the drug concerned, for publication in the *Journal of Pharmaceutical Sciences*. The ready availability of this information affords discriminating medical and pharmaceutical practitioners with an added basis for confidence in the quality of new drug products generally, and of those covered by the monographs particularly. Such monographs will appear on drugs representing new chemical entities for which suitable identity tests and assay procedures are not available in the published literature. The purity and assay limits reported for the drugs and their dosage forms are based on observations made on samples representative of commercial production and are considered to be reasonable within expected analytical and manufacturing variation.

CHLORAL HYDRATE-betaine adduct; $C_7H_{14}Cl_3NO_4$; mol. wt. 282.55. The structural formula of chloral betaine may be represented as



Physical Properties.—Chloral betaine occurs as a white, crystalline powder having a faint aromatic,

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Mead Johnson and Co., Evansville, Ind., has cooperated by furnishing samples and data to aid in the development and preparation of this monograph.