of the method may be increased many-fold. As little as 0.1 mcg. per ml. of apomorphine can be easily estimated. Such a sensitivity would find application in studying the *in vitro* enzymatic degradation of the drug. Presently, the method is being used for studying the metabolism of apomorphine.

SUMMARY

The oxidation of apomorphine hydrochloride with mercuric chloride under standard conditions gives a green-colored product which turns violet when extracted into isoamyl acetate. The absorption peak at 330 mμ exhibited by the colored reaction product, in addition to its peaks in the visible region, has been used for quantitative determination of apomorphine. Catecholamines and other related polyhydroxyphenols in urine do not interfere in the estimation.

Optimum conditions for the reaction have been determined. By simple manipulations, as little as 0.1 mcg. per ml. can be determined. A procedure has been described for the extraction of apomorphine from biological material and solutions containing interfering substances, and the color reaction has been applied to such extracts from human, horse, and rabbit urines.

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Kinetics of the Specific Base-Catalyzed Hydrolysis of Naphazoline*

By MARVIN J. STERN†, LOUIS D. KING, and ARNOLD D. MARCUS‡

The rate of the specific base-catalyzed hydrolysis of naphazoline has been found to be first order with respect to both naphazoline and hydroxyl ion, regardless of whether the naphazoline exists as the protonated or unprotonated species. A theoretical isothermal equation expressing the pseudo-first order specific rate constant as a function of the hydronium ion and hydroxyl ion activities has been derived, and at 25° appears to hold true over a ten million-fold variation in catalyst (hydroxyl ion) concentration. The activation energies and frequency factors for both the hydrolyses of protonated naphazoline and unprotonated naphazoline have been experimentally determined. The reaction involving protonated naphazoline is favored by frequency while the reaction involving unprotonated naphazoline is favored by energy. The frequency effect is the greater so that, at normal temperatures, protonated naphazoline hydrolyzes about one thousand times faster than the unprotonated form. Postulations for the mechanisms of the reactions have been made in light of the kinetic data and a generalized equation for calculating the observed rate constant has been devised.

ALTHOUGH the hydrolytic degradation of esters, amides, imides, and other carbonyl compounds is well recognized, the ability of other structures to degrade through a hydrolytic mechanism is often overlooked. Such oversights may be particularly costly in pharmaceutical research. Excellent examples of such "other" species are the imidazolines. It is, therefore, the purpose of the present communication to describe the kinetics of the specific base-catalyzed hydrolysis of a typical imidazoline—naphazoline.

Naphazoline, 2-(1-naphthylmethyl)-2-imidazoline, has been shown to be relatively stable in acidic or neutral solutions but readily prone to hydrolysis in basic solutions. The first step in this reaction (1) results in the formation of 1-naphthylacetylethylenediamine, which, upon more vigorous treatment (2), undergoes further cleavage to form 1-naphthylacetic acid and ethylenediamine. These reactions can be represented by the following scheme:

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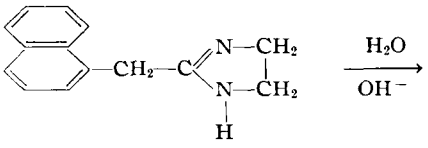
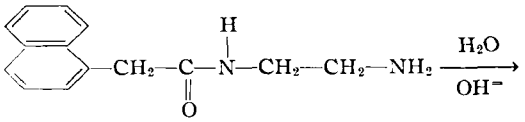
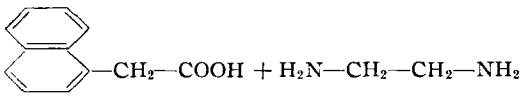
This paper is based on a thesis presented to The Graduate School of Rutgers University by Marvin J. Stern in partial fulfillment of the requirements for the degree of Master of Science.
Portions of this paper are adapted from the honorable mention-winning manuscript submitted by Marvin J. Stern in the Eastern Region, 1958, Lunsford Richardson Pharmacy Award competition.
The authors wish to thank Mr. Jack Cooper of Ciba Pharmaceutical Products, Inc., for his generosity in supplying them with the naphazoline hydrochloride.



Naphazoline



1-Naphthylacetylethylenediamine



1-Naphthylacetic Acid Ethylenediamine

While both steps in the foregoing reaction scheme offer interesting subjects for kinetic studies, it is obvious that only the first step would be of concern in pharmaceutical formulation efforts, since this step is responsible for the disappearance of the intact physiologically active species from its solutions. As a result, it was decided to examine the kinetics of this reaction in detail with the further objective of utilizing the kinetic data in proposing a reaction mechanism.

THEORETICAL CONSIDERATIONS

Naphazoline, like all weak bases, exists in aqueous solutions in both protonated (salt) and unprotonated (base) forms. As a result, any equation describing the rate of hydrolysis must include both potential substrates. The rate expression would therefore be given by

$$-d[\text{Naph}]_T/dt = k_{\text{OH}^-,\,S}\,[\text{OH}^-]^m[\text{Naph}]_S^n + k_{\text{OH}^-,\,B}\,[\text{OH}^-]^{m'}[\text{Naph}]_B^{n'} \quad \text{(Eq. 1)}$$

where the subscripts S, B, and T refer to the salt, base, and total, respectively, and the superscripts are the orders of the reactions with respect to the different species. At constant hydroxyl ion activity, this becomes

$$-d[\text{Naph}]_T/dt = k_S'[\text{Naph}]_S^n + k_B'[\text{Naph}]_B^{n'} \quad \text{(Eq. 2)}$$

where k_S' and k_B' are the pseudo-nth and pseudo-n'th order rate constants for the salt and base forms, respectively. Dividing through by $[\text{Naph}]_T$ gives

$$-\frac{d[\text{Naph}]_T}{[\text{Naph}]_T} = \left(k_S'\,\frac{[\text{Naph}]_S^n}{[\text{Naph}]_T} + k_B'\,\frac{[\text{Naph}]_B^{n'}}{[\text{Naph}]_T}\right)dt \quad \text{(Eq. 3)}$$

If it is assumed that n and n' are both equal to unity (i.e., the reaction is first order with respect to either form of naphazoline), the terms $[\text{Naph}]_S/[\text{Naph}]_T$ and $[\text{Naph}]_B/[\text{Naph}]_T$ will be constant under conditions of constant pH. Integration of Eq. 3 will, therefore, give

$$\log [\text{Naph}]_T = -\frac{(k_S'[\text{Naph}]_S + k_B'[\text{Naph}]_B)t}{2.30\,[\text{Naph}]_T} + \frac{\log [\text{Naph}]_{T,o}}{2.30} \quad \text{(Eq. 4)}$$

where $[\text{Naph}]_{T,o}$ is the initial total naphazoline concentration. The overall reaction would, therefore, follow pseudo-first order kinetics with the specific rate constant (k_{obs}') given by the equation

$$k_{obs}' = \frac{k_S'[\text{Naph}]_S + k_B'[\text{Naph}]_B}{[\text{Naph}]_T} \quad \text{(Eq. 5)}$$

In other words, the observed specific pseudo-first order rate constant is the "weighted average" of the individual salt and base constants.

If the reaction involving the protonated form and the one involving the unprotonated form are of identical order with respect to hydroxyl ion, the relationship

$$k_{\text{OH}^-,\,T} = \frac{k_{\text{OH}^-,\,S}[\text{Naph}]_S + k_{\text{OH}^-,\,B}[\text{Naph}]_B}{[\text{Naph}]_T} \quad \text{(Eq. 6)}$$

can also be written. In solutions of constant hydroxyl ion activity the expression relating the observed rate constant to the true rate constant is given by

$$k_{obs}' = k_{\text{OH}^-,\,T}[\text{OH}^-]^m \quad \text{(Eq. 7)}$$

Substitution of Eq. 6 in Eq. 7 gives, upon taking logarithms

$$\log k_{obs}' = -mp\text{OH} + \log \frac{k_{\text{OH}^-,\,S}[\text{Naph}]_S + k_{\text{OH}^-,\,B}[\text{Naph}]_B}{[\text{Naph}]_T} \quad \text{(Eq. 8)}$$

The relative concentrations of the two forms of naphazoline can be determined by the buffer equation which in this case is

$$\log ([\text{Naph}]_S/[\text{Naph}]_B) = pK_a - pH \quad \text{(Eq. 9)}$$

where K_a is the dissociation constant of the protonated form, the conjugate acid of naphazoline base. Solving Eq. 9 for $[\text{Naph}]_S$ and $[\text{Naph}]_B$ in terms of $[\text{Naph}]_T$ gives

$$[\text{Naph}]_S = \frac{[\text{Naph}]_T[\text{H}^+]}{K_a + [\text{H}^+]} \quad \text{(Eq. 10)}$$

and

$$[\text{Naph}]_B = \frac{[\text{Naph}]_T K_a}{K_a + [\text{H}^+]} \quad \text{(Eq. 11)}$$

Substituting these values in Eq. 8 gives

$$\log k_{obs}' = -mp\text{OH} + \log \left(\frac{k_{\text{OH}^-,\,S}\,[\text{H}^+] + k_{\text{OH}^-,\,B}\,K_a}{K_a + [\text{H}^+]}\right) \quad \text{(Eq. 12)}$$

In solutions of low hydroxyl ion activity naphazoline exists almost entirely in the protonated form. Equation 8 therefore becomes

$$\log k_{obs}' = -mp\text{OH} + \log k_{\text{OH}^-,\,S} \quad \text{(Eq. 13)}$$

Since $k_{\text{OH}^-,\,S}$ is constant at any particular temperature, if Eq. 13 holds, a plot of $\log k_{obs}'$ *versus* pOH will be linear. The slope of the line will be equal to the negative of the order of the reaction with respect to hydroxyl ion. The "y" intercept of any such line will, of course, be equal to $\log k_{\text{OH}^-,\,S}$.

It will be shown in the following sections that the above equations appear to hold true and the assumptions made in their derivations are valid.

EXPERIMENTAL

Determination of Dissociation Constants.—The dissociation constant of naphazoline conjugate acid at each of three temperatures was determined by carefully preparing volumetric solutions of known concentrations of naphazoline hydrochloride and free naphazoline base.[1] This was accomplished by dissolving a known weight of the salt in recently boiled, double-distilled water and adding a known volume of a standard sodium hydroxide solution. Enough sodium chloride to bring the ionic strength to 0.6 was added and the solution was brought to volume with recently boiled, double-distilled water. The pH of the solution was immediately measured at the desired temperature using a Beckman Model H2 pH meter. The pKa value was calculated from Eq. 9.

The values of the dissociation constants so obtained were "semi-classical" values, i.e., based on hydrogen ion activities and all other values as concentrations. Since all of the measurements and calculations involved are actually in these units, the "semiclassical" dissociation constants are the proper values to use. Due to the presence of concentration terms, these constants had to be measured under conditions of the same ionic sterngth as the experimental naphazoline solutions in order to be meaningful.

Buffer Systems.—Buffer solutions were utilized in order to keep the hydroxyl ion activities constant during the kinetic runs. To obviate any error due to the effect of ionic strength on the reaction rates, all buffers were made to have ionic strengths of exactly 0.6 by adjustment with sodium chloride. The buffers were prepared by blending a solution of the base (or acid) at $\mu=0.6$ and a solution of its salt at $\mu=0.6$ until the desired pH was obtained. The solutions were made 0.2 *M* with respect to total buffer constitutents wherever the solubility permitted. The ionic strength figure of 0.6 was chosen since this represents the ionic strength of a 0.2 *M* solution of a 1:2 or 2:1 electrolyte.

Buffers were prepared to cover the pH range of approximately 6 to 14. The buffer types, in order of increasing pH, were: triethanolamine-triethanolamine hydrochloride, sodium borate-boric acid, sodium carbonate-sodium bicarbonate, diethylamine-diethylamine hydrochloride, and for very high pH's (above 12), dilute solutions of sodium hydroxide were used. The usual calcium or barium hydroxide could not be used due to the presence of sodium bicarbonate in the assay procedure (3). Phosphate salts also could not be used in buffers for this work since their presence seemed to prevent the colored complex developed in the assay from adhering to Beer's law, even in very dilute solutions.

The highest pH obtainable was 13.48 at 25°, corresponding to a 0.6 *M* sodium hydroxide solution. The fact that the buffer solutions were all made to have ionic strengths of 0.6 precluded the use of more concentrated hydroxide solutions.

[1] Due to the low solubility of the naphazoline base, the solutions were made on the order of 2.5×10^{-3} *M* with respect to the salt and base.

An important consideration in the use of buffer solutions is the fact that the hydroxyl ion activity changes with temperature by virtue of the thermal change of the value of the ion product of water. The change in hydroxyl ion activity is especially evident in the case of acid-salt buffers where the hydronium ion activity is kept relatively constant and changes in the ion product of water are reflected, for the most part, in changes in the activity of hydroxyl ion. Even in base-salt buffers, however, there is an appreciable thermal change in hydroxyl ion activity. The common practice of ignoring these changes was considered to be highly inaccurate, since calculations show that in a reaction first order with respect to hydroxyl ion, a change of as little as 0.2 pOH units represents an error of about 60% in the specific rate constant. This error is even greater in reactions of higher order with respect to hydroxyl ion.

In order to determine the correct values of the hydroxyl ion activities, the pH of each buffer was measured at the temperatures at which the kinetic runs were made. The pH's were subtracted from the thermodynamic pKw's at the temperatures in question (4) to give the pOH's.

Kinetic Runs.—One hundred milligrams of naphazoline hydrochloride was placed in a 100-ml. glass-stoppered volumetric flask, enough buffer solution added to make 100 ml., and the solution was filtered. The flask was placed in a constant temperature oil bath. After thermal equilibrium had been attained, a 5-ml. sample was removed from the flask, delivered into a glass vial, stoppered, and plunged into an ice-water mixture. In the case of high pH systems, where sudden cooling was not sufficient to quench the reaction, the vial contained a predetermined amount of hydrochloric acid sufficient to give a final pH of about 6. This sample was arbitrarily designated as the "zero-hour" sample. Additional samples were removed and quenched after appropriate time intervals. All samples were refrigerated until the time of assay.

Routine analyses for residual naphazoline were carried out by the colorimetric method of Slack and Mader (3). Prior to color development, the solutions were diluted with sufficient buffer so that the absorbance of the colored complex did not exceed 0.4. At higher absorbances, the buffer constituents appear to cause deviations from Beer's law.

The analytical procedure used to verify the stoichiometry of the reactions was the partition chromatographic procedure of Schwartz, Kuramoto, and Malspeis (2), as modified by Stern (5).

All volumetric withdrawals from the refrigerated samples were done with the vials at 0°.

RESULTS AND DISCUSSION

Dissociation Constants.—The pKa values of naphazoline conjugate acid were determined as described in the experimental section. Three solutions of different base:salt ratios were used for the determination of each pKa value and the average was taken. These "semiclassical" pKa values at 0.6 inonic strength are given in Table I. These values are in very good agreement with the results of Hall and Sprinkle (6) who found that the pKa's of the conjugate acids of bases of about the strength of

TABLE I.—SEMICLASSICAL DISSOCIATION CONSTANTS OF NAPHAZOLINE CONJUGATE ACID AT $\mu = 0.6$

Temperature, °C.	pKa
25.0	10.35 ± 0.02
35.0	10.13 ± 0.02
45.0	9.92 ± 0.03

naphazoline showed a linear decrease of about 0.02 for each degree increase in temperature.

Verification of the Stoichiometry of the Reaction.—In order to show that hydrolysis was the only degradative pathway under the conditions employed in the kinetic runs, it was necessary to verify the stoichiometry of the reaction. A kinetic run in pOH 4.55 borate buffer at 65° was chosen for this since this pOH and temperature were considered to be most representative of the conditions employed in the subsequent kinetic runs. This buffer also corresponded in pH to the internal phase of the Celite 545 partition column employed in the assay (2, 5).

The samples were assayed for naphazoline, 1-naphthylacetylethylenediamine, and 1-naphthylacetic acid. The results are illustrated graphically in Fig. 1. This plot clearly shows that hydrolysis is the only significant degradative pathway under the conditions employed since all of the naphazoline was accounted for and the total molar concentration of naphazoline plus its hydrolytic degradation products was constant. The 1-naphthylacetylethylenediamine did not hydrolyze to any significant extent to form 1-naphthylacetic acid.

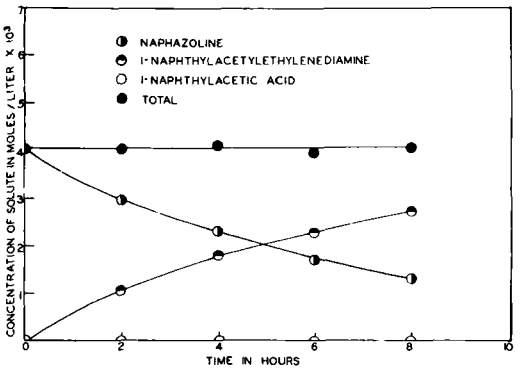


Fig. 1.—Verification of the stoichiometry of the reactions.

Effect of Naphazoline Concentration.—In Fig. 2, the residual naphazoline concentrations from the kinetic run in pOH 4.55 buffer at 65° are plotted logarithmically against time. From the straight line obtained, it appears that the rate of hydrolysis exhibits a first order dependency on the naphazoline concentration. The fact that the same line was obtained when calculated from direct analysis of the residual naphazoline or from the analyzed concentrations of the decomposition products further illustrates the stoichiometry of the degradation. All of the subsequent kinetic runs throughout the entire temperature and pH ranges that were investigated showed similar first order dependencies. From the theoretical considerations presented, this would certainly indicate that the reaction is first order with respect to naphazoline regardless of whether the imidazoline exists as the protonated or unprotonated species.

Effect of Hydroxyl Ion Activity.—In Fig. 3 the logarithms of the pseudo-first order rate constants have been plotted against pOH for the range where naphazoline exists almost exclusively in the protonated form. Straight lines with slopes of −1.00 have been obtained, which, from Eq. 13, indicates that the rate of hydrolysis of naphazoline is first order with respect to hydroxyl ion. The plots have

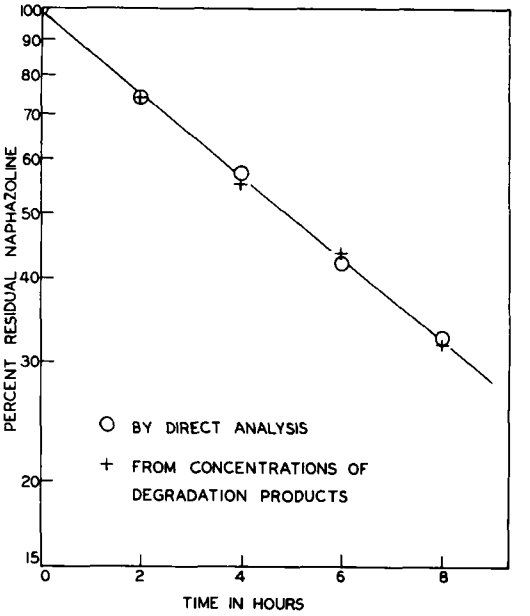


Fig. 2.—Kinetic run at 65.0° in 0.1 *M* borate buffer; $\mu = 0.6$; pOH = 4.55.

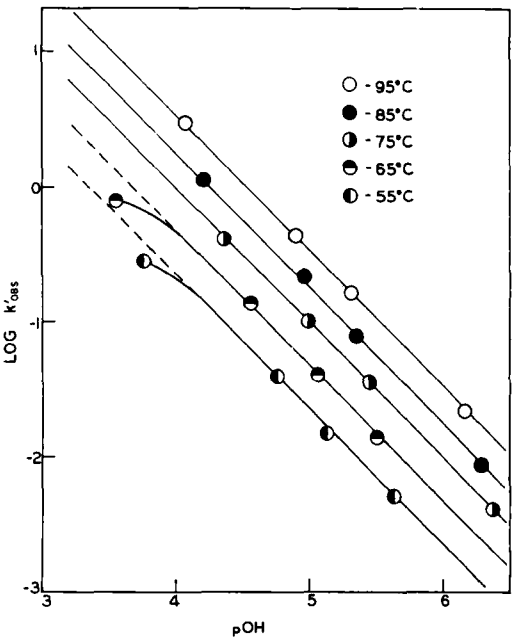


Fig. 3.—Effect of hydroxyl ion activity on the rate of hydrolysis.

been extrapolated to the "y" intercepts to obtain the logarithms of the second order specific rate constants which appear in Table II.

It can be seen in Fig. 3 that the points in the vicinity of pOH 3.5–3.8 do not fall on the lines, but below them. These deviations are in accord with the theoretical considerations governing high pH (low pOH) systems where the presence of the unprotonated form of naphazoline is greatly increased. All other things being equal, the unprotonated base would react at a slower rate than the protonated salt form due to the coulombic attraction between the latter and hydroxyl ions.

The effect of hydroxyl ion on the rate of hydrolysis at 25° in the pOH range 0–8 is given in Fig. 4. While the points were experimentally determined, the line was not fitted to the experimental data, but instead, was drawn to represent the relationship expressed by Eq. 12. The values of $k_{OH^-, S}$ and $k_{OH^-, B}$ used in solving Eq. 12 were 182 liters moles^{-1} hours^{-1} and 0.187 liters moles^{-1} hours^{-1}, respectively. The value of $k_{OH^-, S}$ was obtained by extrapolation from results at higher temperatures. The kinetic run at 25° at pH 12.98 was used for the determination of $k_{OH^-, B}$.

Inasmuch as the derivation of Eq. 12 required that the hydrolyses of both protonated and unprotonated naphazoline have the same kinetic dependency on hydroxyl ion, the excellent agreement shown in Fig. 4 between the experimental results and theoretical equation indicates that this requirement was met. Since it has been shown that the hydrolysis of protonated naphazoline is first order with respect to hydroxyl ion, the hydrolysis of unprotonated naphazoline must also be first order with respect to this ion.

TABLE II.—SPECIFIC RATE CONSTANTS AT ELEVATED TEMPERATURES FOR THE HYDROLYSIS OF PROTONATED NAPHAZOLINE

Temperature ° C.	$k_{OH^-, S}$, liters moles^{-1} hours^{-1}
95	3.4×10^4
85	1.8×10^4
75	9.8×10^3
65	4.7×10^3
55	2.2×10^3

The temperatures were controlled to ±0.05° or better.

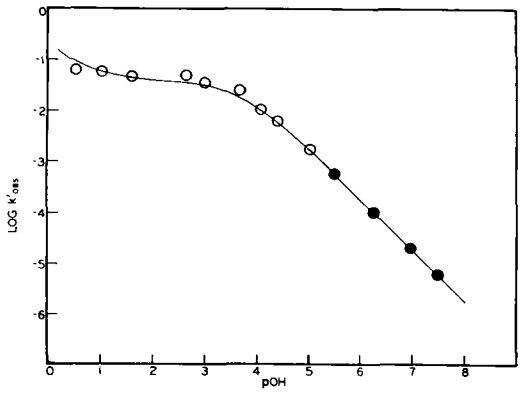


Fig. 4.—Effect of hydroxyl ion activity on the rate of hydrolysis at 25.0°. The line represents the relationship expressed by Eq. 12. The open circles are experimental points determined at 25.0°. The solid circles are experimental points determined at other temperatures and extrapolated to 25.0°.

Temperature Dependencies of the Reactions.—The Arrhenius-type plots which appear in Figs. 5 and 6 show the temperature dependencies of the reactions. Difficulty was encountered during attempts to determine the effect of temperature on the rate of hydrolysis of the unprotonated form of naphazoline. The difficulty evolved from the fact that at normal temperatures the specific rate of hydrolysis of the protonated form is about one thousand times faster than that of the unpro-

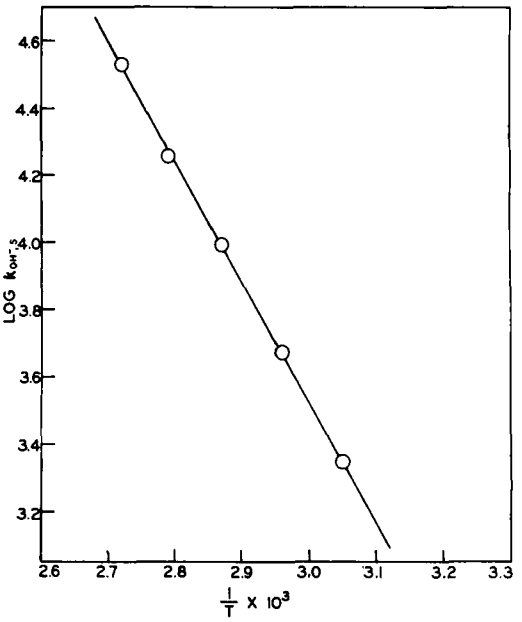


Fig. 5.—Effect of temperature on the rate of hydrolysis of protonated naphazoline.

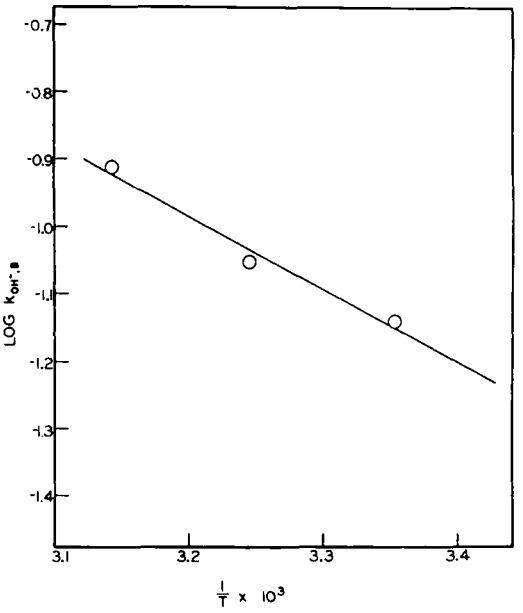


Fig. 6.—Effect of temperature on the rate of hydrolysis of unprotonated naphazoline.

tonated form. Thus, even at pH 13.48, the highest pH that could be used, where the concentration of the unprotonated form is over thirteen hundred times greater than the concentration of the protonated form, the hydrolysis of the latter still accounts for over two-fifths of the overall degradation rate. Any rate constant for the hydrolysis of the unprotonated form derived under such conditions is, at best, an approximation, as would be any thermodynamic or kinetic value calculated from such rate constants. It was felt, however, that such approximations would still yield useful information. In Fig. 6, the regression line was drawn using the method of least squares (7).

The activation energy and frequency factor for the hydrolysis of protonated naphazoline have been calculated from the plot in Fig. 5. These were found to be 16.4 kilocalories per mole and 1.91×10^{14} liters moles^{-1} hours^{-1}, respectively. The values of the same parameters for the hydrolysis of unprotonated naphazoline, determined from Fig. 6, were found to be 4.9 kilocalories per mole and 3.0×10^{2} liters moles^{-1} hours^{-1}, respectively.

A generalized equation for the specific rate constant for the base-catalyzed hydrolysis, modeled after the Arrhenius equation, can be written as

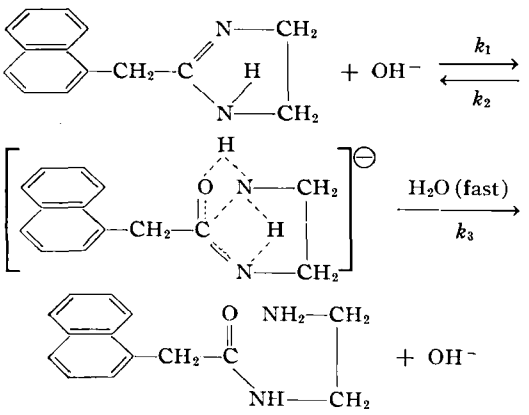
$$k_{obs}' = \frac{(1.91 \times 10^{14}\, e^{-16,400/1.99\tau}\,[\text{Naph}]_S + 3.0 \times 10^{2}\, e^{-4,900/1.99\tau}\,[\text{Naph}]_B)[\text{OH}^-]}{[\text{Naph}]_T} \quad \text{(Eq. 14)}$$

where τ is the absolute temperature. Substituting the equivalents previously derived for $[\text{Naph}]_S$ and $[\text{Naph}]_B$ (see Eqs. 10 and 11) gives

$$k_{obs}' = \frac{1.91 \times 10^{14}\, e^{-16,400/1.99\tau}\, K_w + 3.0 \times 10^{2}\, e^{-4,900/1.99\tau}\, K_a[\text{OH}^-]}{K_a + [\text{H}^+]} \quad \text{(Eq. 15)}$$

Mechanisms of the Reactions.—While the kinetic data which have been presented are valuable in applications such as stability evaluation, they have much more fundmental utility. Kinetic data offer one important means of testing the validity of reaction mechanisms, and it is with the knowledge of mechanisms that the ultimate in predictions can be made. For this reason, mechanisms for the hydrolysis of naphazoline, which are in accord with the kinetic data, are proposed.

The hydrolysis of naphazoline base can follow the course represented by



While the exact nature of the intermediate cannot be unambiguously proved, the depicted structure represents a hybrid or compilation of the resonant forms that can be postulated. A model of the intermediate, constructed from Godfrey Molecular Models (8), indicates that the proposed structure is feasible.

The second step in the reaction, replacement of the hydroxyl group of the water, would be very fast. In other words, almost as soon as the unstable intermediate is formed, it reacts with the water. The value of k_3, therefore, would be much larger than that of either k_1 or k_2. Applying the steady state treatment to these reactions gives the following rate equation:

$$-d[\text{Naph}]/dt = k_1\left(1 - \frac{k_2}{k_2 + k_3[\text{H}_2\text{O}]}\right)[\text{Naph}][\text{OH}^-] \quad \text{(Eq. 16)}$$

Since in dilute aqueous solutions, all of the terms within the parentheses are constant, they can be combined with k_1 to give an overall second order expression:

$$-d[\text{Naph}]/dt = k_{\text{OH}^-}[\text{Naph}][\text{OH}^-] \quad \text{(Eq. 17)}$$

The value of k_{OH^-} should be very close to that of k_1 since the term $k_2/(k_2+k_3[H_2O])$ would be very small due to the large values of k_3 and $[H_2O]$.

According to Eq. 17, the reaction would be first order with respect to both naphazoline and hydroxyl ion. The experimental data appear to satisfy this requirement.

The fact that, in the proposed mechanism, hydroxyl ion is both a reactant and a product would make the reaction a true specific base-catalyzed hydrolysis.

Although the depicted mechanism is for the hydrolysis of unprotonated naphazoline, a very similar scheme can be proposed for the hydrolysis of protonated napthazoline.

The Arrhenius frequency factor of 1.91×10^{14} liters moles^{-1} hours^{-1} for the reaction involving protonated naphazoline is a "normal" value for a bimolecular reaction in solution as calculated from collision theory (9). Upon cursory consideration, one might expect this parameter to be considerably greater by virtue of the fact that the reacting entities are of opposite electrical charge and, thus, exhibit coulombic attraction. This, however, is offset by two factors. First, in ionic interactions, the hydration shell has a restraining effect on the ions and will thus slow down the rate of collision. Second, the proposed mechanism of the reaction is such that the hydroxyl ion can attack from only one side of the naphazoline molecule, *viz.*, the side in line with the double-bonded imino nitrogen atom. For this reason, not every collision between ions of the proper energy will result in a reaction and the frequency factor will be lowered. The much lower

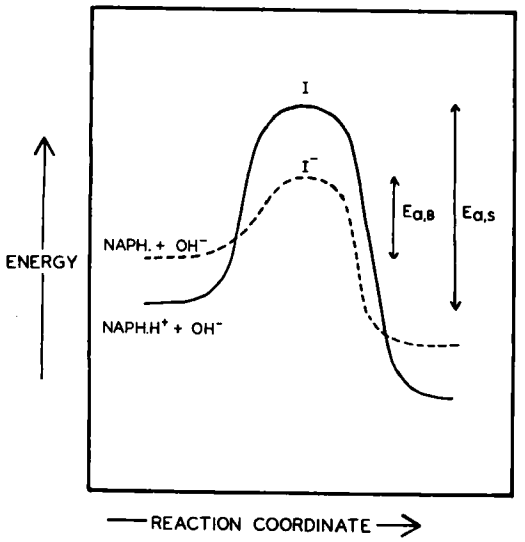


Fig. 7.—Energetics of the reactions.

frequency factor (3.0×10^2 liters moles^{-1} hours^{-1}) for the reaction involving unprotonated naphazoline is also to be expected since the frequency of collision for an ion and a neutral molecule would be much lower than for two oppositely charged ions.

The relative activation energies can best be explained by the energy diagram in Fig. 7. Naphazoline conjugate acid, being charged, is more stable in solution than is the uncharged naphazoline base. Similarly, the activated complex formed in the reaction involving the free base has a charge and is more stable in solution than the uncharged intermediate in the reaction involving the conjugate acid (protonated) form. Since the activation energy is the difference in energy between the reactants and the activated complex, this parameter for the free base reaction would be considerably less than for the conjugate acid reaction. The experimental values of 4.9 kilocalories per mole and 16.4 kilocalories per mole, respectively, agree with the above considerations.

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Analysis of Adrenergic Drug Action*

By R. S. McCUTCHEON† and RAYMOND P. AHLQUIST

Three analogs of arterenol were comparatively studied on several effector systems in dogs. The amines were: (*a*) ethylnorepinephrine (butanephrine), (*b*) isoproterenol (Isuprel), and (*c*) the N-isopropyl derivative of ethylnorepinephrine (Win-3046). These amines were compared to levarterenol and epinephrine as to potency and effect on arterial pressure, heart rate, intestinal motility, splenic capsule, and urine output. The effects on the splenic capsule best illustrates the general relationships found. Epinephrine, levarterenol, and ethylnorepinephrine produce primary splenic contraction; this effect is prevented by adrenergic blockade and is not modified by ganglionic blockade. Compounds (*b*) and (*c*), and (*a*) to some extent, induce a reflex splenic contraction by their depressor action. This effect is prevented by either adrenergic or ganglionic blockade. When administered simultaneously with epinephrine, (*b*) and (*c*) diminish the splenic contraction produced by the epinephrine.

THE ADRENERGIC RECEPTOR is the primary site of action of the adrenergic neurohormone and adrenergic drugs. It has been proposed that the adrenergic receptor occurs in two different forms (1). As far as smooth muscle is concerned, one of these receptors, known as the *alpha* receptor serves primarily excitatory responses. The other receptor, known as the *beta* receptor, subserves primarily inhibitory responses. Some adrenergic drugs activate both receptors. Therefore, it would be desirable to have a convenient method for determining the differential potency on these two receptors. To investigate such a method, a comparative study in the anesthetized dog has been carried out using epinephrine, levarterenol, isoproterenol, ethylnorepinephrine, and the N-isopropyl derivative of ethylnorepinephrine (Win-3046).

* Received July 20, 1959, from the Departments of Pharmacology and Physiology, Medical College of Georgia, Augusta.
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EXPERIMENTAL

Method.—Mongrel dogs of either sex weighing 10 to 15 Kg. were anesthetized with pentobarbital sodium, 10 to 15 mg./Kg., administered intravaneously thirty minutes after a subcutaneous injection of morphine sulfate, 10 mg./Kg. The arterial pressure was recorded from either the femoral or carotid artery by means of a mercury manometer or a Statham transducer. The contraction of the