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ACKNOWLEDGMENTS AND ADDRESSES

Received April 27, 1970, from the *School of Pharmacy, University of California, San Francisco, CA 94122*

Accepted for publication April 30, 1971.

Abstracted from a dissertation submitted by W. L. Chiou to the Graduate Division, University of California, San Francisco Medical Center, in partial fulfillment of Doctor of Philosophy degree requirements.

Supported in part by a grant-in-aid from the funds of the Academic Senate Committee on Research, San Francisco Division, University of California.

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Hydrolysis of Benzothiadiazines

J. A. MOLLICA, C. R. REHM, J. B. SMITH, and H. K. GOVAN

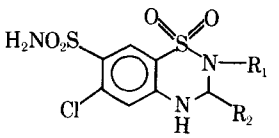
Abstract □ The hydrolysis of hydrochlorothiazide and two other hydrothiazides was studied as a function of pH. Reversible kinetics were observed for the hydrolytic reaction, and a bell-shaped pH–rate profile was obtained. The equilibrium constant, however, was relatively independent of hydrogen-ion concentration. The hydrolytic rate constants and the equilibrium constants for the overall reaction were evaluated. General catalysis was checked at several pH values, utilizing acetate and imidazole buffers, and no significant buffer catalysis was observed. The bell-shaped pH–rate profiles cannot be explained completely by the ionization of reactants, but they can be interpreted by postulating the presence of an imine intermediate that undergoes attack by water or hydroxide to form a carbinolamine which subsequently decomposes to products.

Keyphrases □ Benzothiadiazines—hydrolysis, mechanism, pH–rate profiles □ Hydrochlorothiazide, 3- and 2-ethyl analogs—hydrolysis, mechanism, pH–rate profiles □ Hydrolysis, rates and mechanism—benzothiadiazines

Benzothiadiazines form one of the most important classes of compounds used as diuretics. They have been divided into two main categories: "thiazides," which are 2*H*-1,2,4-benzothiadiazine-1,1-dioxides, and "hydrothiazides," which are 3,4-dihydro-[2*H*]-1,2,4-benzothiadiazine-1,1-dioxides. The former is represented by chlorothiazide, which is formed by condensing 5-chloro-2,4-disulfamylaniline (hereafter referred to as "disulfonamide") with formic acid; the latter is represented by hydrochlorothiazide, which is formed by condensing disulfonamide with formaldehyde (1).

Although these compounds form an important class of drugs, few publications concerning their routes and mechanisms of degradation have appeared. Yamana *et al.* (2) reported on the hydrolysis of chlorothiazide, and the stability of this compound was also studied by Charnicki *et al.* (3) and Baer *et al.* (4). Hydrothiazides, however, have not been studied in detail. Previous reports from this laboratory considered the analysis of hydrochlorothiazide (5) and presented the pH–rate profile for its hydrolysis (6). Yamana *et al.* (7) also published a similar report covering the pH–rate profile and reported on the hydrolysis in 1 *N* NaOH (8).

The study of the hydrolysis of hydrochlorothiazide was extended in this laboratory to explore the validity of the hypotheses that the reaction is reversible and that the extent of reaction is independent of pH. In addition, factors that influence the rate of reaction were investigated in order to postulate a possible mechanism for the hydrolysis. The present article is an extensive report on the hydrolysis of hydrochlorothiazide and its 3-ethyl (Su 9604) and 2-ethyl (Su 6835) analogs.



hydrochlorothiazide	$R_1 = R_2 = H$
N-ethyl hydrochlorothiazide (Su 6835)	$R_1 = C_2H_5$, $R_2 = H$
C-ethyl hydrochlorothiazide (Su 9604)	$R_1 = H$, $R_2 = C_2H_5$

EXPERIMENTAL

Materials—The purity of hydrochlorothiazide, m.p. 268°, was determined by phase-solubility analysis. All material used in these studies had a minimum assay of 99.4% by this method. The purity of 5-chloro-2,4-disulfamylaniline, m.p. 251–252°, was also determined by phase-solubility analysis. All material used had a minimum assay of 99.2% by this method. The following compounds were prepared in the research laboratories at CIBA and were previously reported (1, 9)[1]:

1. 6-Chloro-2-ethyl-3,4-dihydro-7-sulfamyl-2*H*-1,2,4-benzothiadiazine-1,1-dioxide [Su 6835], m.p. 190–191°.

Anal.—Calc. for $C_9H_{12}ClN_3O_4S_2$: C, 33.18; H, 3.71; N, 12.90. Found: C, 32.87; H, 3.70; N, 13.18.

2. 6-Chloro-3-ethyl-3,4-dihydro-7-sulfamyl-1,2,4-benzothiadiazine-1,1-dioxide [Su 9604], m.p. 257°.

Anal.—Calc. for $C_9H_{12}ClN_3O_4S_2$: C, 33.18; H, 3.71; N, 12.90. Found: C, 33.06; H, 3.92; N, 13.15.

[1] The authors thank Dr. L. Werner for generously providing these compounds.

Formaldehyde, 37%, reagent grade, was assayed according to USP XVI; ethylene glycol monomethyl ether, reagent grade, was used directly.

Buffers—Reagent grade chemicals were used without further purification in the preparation of buffer solutions, with the exception of imidazole which was recrystallized from benzene, using Norit as a decolorizing adsorbent, m.p. 89–90°. The following buffers were used: pH 1–2.5, hydrochloric acid; 2.5–4, formate; 4–6, acetate; 5–7.5, imidazole; 5–11, phosphate; 9–10, borate; and 11–13, hydroxide. All pH measurements of buffer solutions were made at ambient temperatures, except for imidazole buffers which were also measured at 60°. The meter–electrode system was standardized against the standard buffers recommended by Bates (10).

Ionization Constants—The dissociation constants of hydrochlorothiazide were determined by potentiometric titration, using a Radiometer titrator and a thermostated titration cell at 60°. Calculations following the method of Noyes (11), for substances with two ionizing groups with pKa values less than 2.7 units apart, were made. Values of 8.6 and 9.9 were obtained, respectively, for pKa_1, and pKa_2. The Merck Index gives values of 7.9 and 9.2, respectively (12). Spectrophotometric measurements yielded only one pKa value for the compounds: hydrochlorothiazide, 8.7; *N*-ethyl hydrochlorothiazide, 9.5; and *C*-ethyl hydrochlorothiazide, 8.8, all at 25°.

Equipment—The pH values were measured with a Radiometer model 25SE meter, using a K401 calomel electrode and a G202B glass electrode. A Radiometer titrator type TTTla and titragraph type SBR2C were used for the determination of the ionization constants by potentiometric titration. Spectrophotometric measurements were made using a Beckman model DU, and spectra were obtained with a Cary model 11 or 14 spectrophtometer. Constant-temperature baths were maintained to within 0.05° of specified temperatures.

Procedures—Several procedures were utilized to monitor the reactions:

1. Colorimetric determination of 5-chloro-2,4-disulfamylaniline by reaction with nitrous acid and coupling with chromotropic acid, according to the previously published procedure (5), was the primary method used in the kinetic studies.

2. There was sufficient difference in the UV absorption spectra of hydrothiazides and disulfonamide so that rate data could be obtained by monitoring the decrease in absorbance at constant wavelength (λ = 272 nm.) as a function of time.

3. Formaldehyde was determined colorimetrically with phenylhydrazine and potassium ferricyanide, utilizing a modified procedure of Tanenbaum and Brecker (13).

The determination of formaldehyde was the primary method employed to monitor the hydrolysis of *N*-ethyl hydrochlorothiazide. In addition, the 2-(*N*-ethylsulfamyl)-4-sulfamyl-5-chloroaniline produced upon hydrolysis was determined by the colorimetric procedure. This yields a colored compound with essentially the same spectrum as that produced by the unsubstituted disulfonamide. Rate constants calculated by following the appearance of formaldehyde or of the aniline agree to within 5%.

Pseudo-first-order rate constants were calculated by taking the initial slopes of plots of log $(a - x)$ *versus* time, where a is the initial concentration of hydrothiazide and x is the concentration of disulfonamide produced, and/or from plots of:

$$\log \frac{1 - (1 - Xe)(1 - X)}{(1 - X) - (1 - Xe)} \textit{ versus } \text{time}$$

where X is the fraction reacted at time, t, and Xe is fraction reacted at equilibrium. In alkaline solution, rate constants were evaluated by determining the initial rate of formation of disulfonamide produced over the first few percent of reaction.

RESULTS AND DISCUSSION

The hydrolysis of hydrochlorothiazide and *C*-ethyl hydrochlorothiazide approaches a state of equilibrium which is invariant with pH. The fraction reacted, Xe, defined as $1 - C_e/C_0$, where C_e and C_0 are, respectively, the equilibrium and initial concentration of hydrothiazide, as a function of pH is given in Table I. The extent of reaction does vary with initial concentration, yet the equilibrium constant remains invariant within the experimental error as expected. *N*-Ethyl hydrochlorothiazide hydrolyzes very slowly, and it was not possible to obtain equilibrium values in all cases. For example, at pH 7.9 the compound is only 12% hydrolyzed after 2 months at 60°.

Table I—Extent of Reaction and Equilibrium Constant as a Function of pH and Concentration

Compound	pH	C_0(× 10^4 *M*)[a]	*Xe*[b]	*K*(× 10^4 *M*)[c]
Hydrochlorothiazide	1.50	6.74	0.41	1.9
	4.61	6.65	0.41	1.9
	4.62	16.8	0.28	1.9
	4.60	67.2	0.16	2.2
	7.41	6.72	0.40	1.8
	8.18	67.2	0.16	2.0
	4.38[d]	6.78	0.42	2.1
C-Ethyl hydrochlorothiazide	3.60	6.14	0.80	20
	5.29	6.14	0.80	20
	7.75	6.14	0.81	21
	9.80	6.14	0.79	18
N-Ethyl hydrochlorothiazide	3.56	6.14	0.43[e]	—
	4.44	6.14	0.43	—
	5.86	6.14	0.50	—

[a] Initial concentration of hydrothiazide. [b] $Xe = 1 - C_e/C_0$, where C_e and C_0 are, respectively, the equilibrium and initial concentration of hydrothiazide. [c] K = (aldehyde) (disulfonamide)/(hydrothiazide). [d] Formation of hydrochlorothiazide from equimolar (6.78×10^{-4}) concentrations for disulfonamide and formaldehyde. [e] Extent of reaction calculated at 1728 hr.

Formaldehyde is essentially completely hydrated in aqueous solution; however, its rate of dehydration should not be rate controlling under the conditions of these experiments (14). In alkaline solution, however, the disproportionation of formaldehyde into methanol and formic acid becomes significant and the reaction can be driven to completion in basic media. Hence, rate constants for alkaline hydrolysis were obtained from the initial slopes of plots of disulfonamide produced as a function of time (Fig. 3), and at other pH

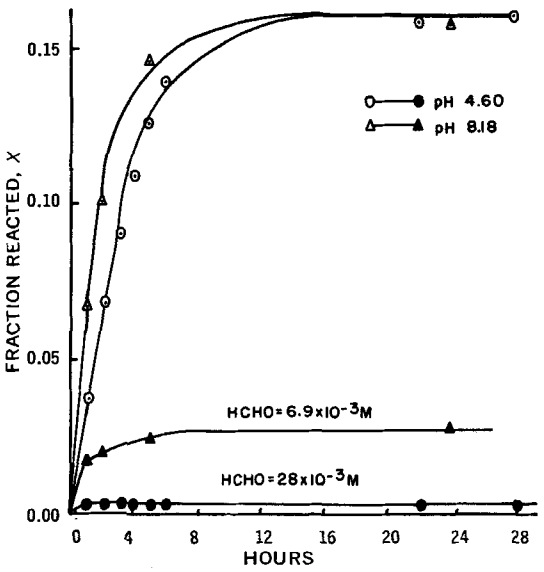


Figure 1—*Plot showing the extent of reaction,* X, *for the hydrolysis of hydrochlorothiazide in the presence of an added equimolar amount and an excess of formaldehyde.* $C_0 = 6.71 \times 10^{-3}$ M *hydrochlorothiazide;* T = 60°.

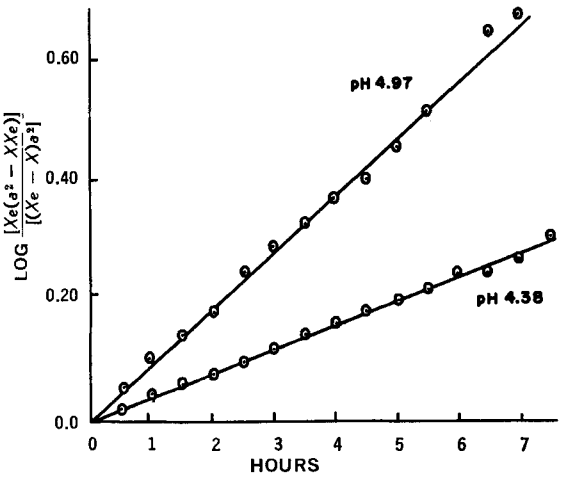


Figure 2—*Plots of the integrated equation for the formation of hydrochlorothiazide from equimolar concentrations of disulfonamide and formaldehyde at two pH values.* $C_0 = 6.78 \times 10^{-4}$ M; T = 60°.

values they were obtained by plotting the data as described previously. Figures 4 and 5 show typical plots of the integrated equation.

Although propionaldehyde does not undergo the Cannizzaro reaction, it can undergo base-catalyzed condensation. However, hydrolysis of *C*-ethyl hydrochlorothiazide was only studied in the pH range 3–10 and was carried to equilibrium at all pH values studied.

The effect of ionic strength on the hydrolysis of hydrochlorothiazide at pH 4.6 was determined. Variation of ionic strength from 0.2 to 1.3 had no effect on the rate of reaction when the rate constants, $k_{obsd.}/a_{OH^-}$, were compared.

Arrhenius plots were made at pH 1.47 and 4.01. The activation energy was 25.3 kcal. at pH 1.47 and 30.6 kcal. (18.7 kcal. when corrected for the ΔH of water) at pH 4.01. This may be compared to the value of 27.7 kcal. reported for the hydrolysis of hydrochlorothiazide in 1 *N* sodium hydroxide solution (8). The extent of reaction is relatively insensitive to temperature, as can be seen from the data in Table II. The rate constant extrapolated to 25° is approximately 4×10^{-5} hr.$^{-1}$ at pH 4. This is in fair agreement with a value calculated utilizing the equilibrium constant of 2×10^{-4} M^{-1} and a half-life of 70 min. for the reaction of disulfonamide with 0.1 *M* formaldehyde at 25° (the reverse reaction follows pseudo-first-order kinetics in the presence of excess formaldehyde).

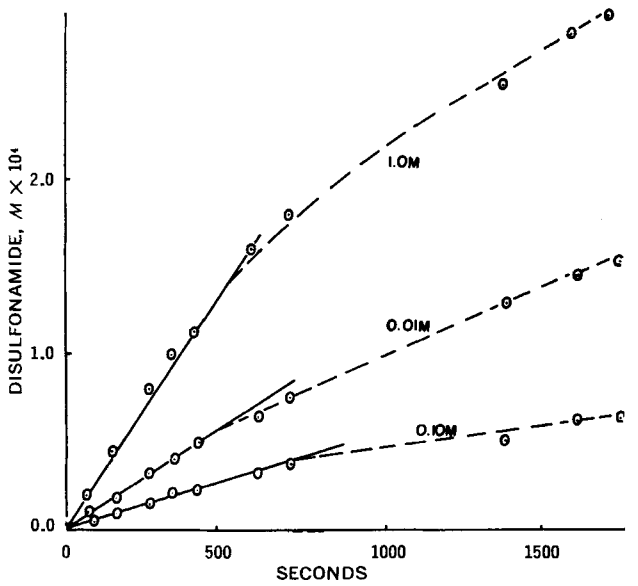


Figure 3—*Concentration of 5-chloro-2,4-disulfamylaniline produced from the hydrolysis of hydrochlorothiazide at several hydroxide-ion concentrations;* T = 60°. *Rate constants were obtained from the initial slopes.*

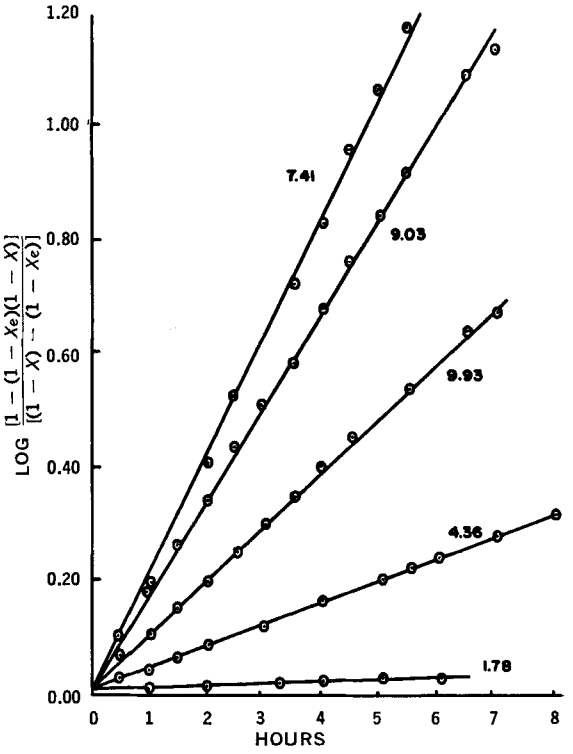


Figure 4—*Plot of the integrated form of the rate equation for an equilibrium reaction of the type* A = B + C *for the hydrolysis of hydrochlorothiazide at several pH values;* T = 60°.

The data in Table III indicate that there is only a slight buffer catalysis. This is in accord with observations made by Yamana *et al.* (7). Analogous reactions such as the base-catalyzed decomposition of formocholine chloride (15) and the hydrolysis of benzylidene-*tert*-

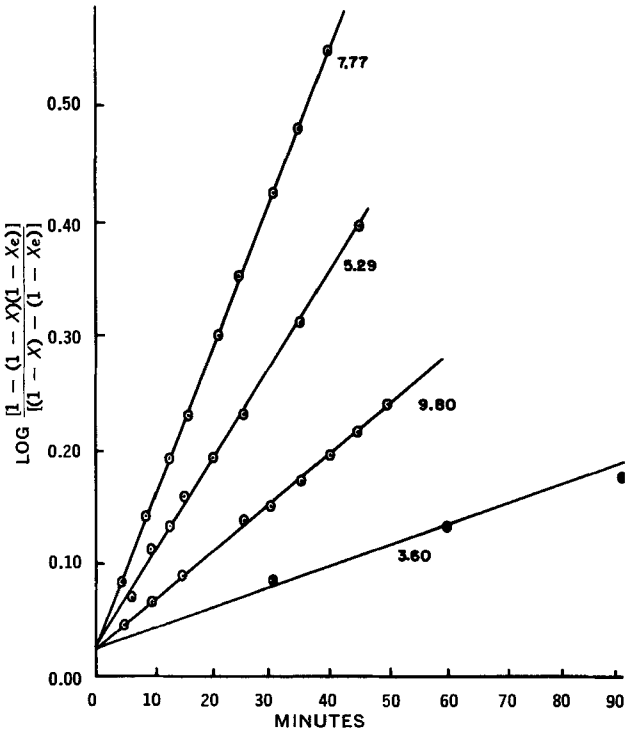


Figure 5—*Plot of the integrated form of the rate equation for an equilibrium reaction of the type* A = B + C *for the hydrolysis of* C*-ethyl hydrochlorothiazide at several pH values;* T = 60°.

Table II—Rate Constants and Extent of Reaction for Hydrolysis of Hydrochlorothiazide at Several Temperatures and at pH 1.47 and 4.01

Temperature	pH 1.47 10^3k, hr.$^{-1}$	pH 1.47 Xe^a	pH 4.01 10^3k, hr.$^{-1}$	pH 4.01 Xe^a
45°	1.46	0.35	1.20	0.44
53°	3.98	0.37	4.03	0.38
62°	10.8	0.43	14.5	0.42
70°	26.7	0.47	42.3	0.47

[a] $C_0 = 6.7 \times 10^{-4}$ *M*.

butylamine (16) are also not subject to buffer catalysis. The change in the extent of reaction for the hydrolysis of hydrochlorothiazide with imidazole buffers was at first surprising. Although one expects a primary amine or other carbonyl reagent to react readily with formaldehyde [it was reported (5) previously that hydroxylamine will bring the reaction to completion by reacting with the formaldehyde produced], the apparent reaction of formaldehyde with a secondary and/or a tertiary amine was at first unexpected. However, Kallen and Jencks (17) reported the formation of protonated hydroxymethylamines of secondary amines and their data strongly suggest that imidazole reacts with formaldehyde. The increase in extent of reaction, therefore, with both increasing buffer concentration and pH agrees with the conclusions in their study regarding the complex equilibrium between imidazole and formaldehyde.

The bell-shaped pH–rate profiles obtained (Fig. 6) are relatively complex and not amenable to simple interpretation. The discussion will be of the pH–rate profile for hydrochlorothiazide, the compound is studied in greatest detail. However, the behavior of the other two compounds is analogous.

Although the inflection point on the descending portion of the curve is close to the ionization value for hydrochlorothiazide (hydrothiazides and the disulfonamides behave as monoprotic or diprotic acids, depending on the extent of substitution with pKa values in the range 8–10), the left inflection point (pH 4.5) does not correspond to a dissociation constant. The bell-shaped profile must be, therefore, an expression of the kinetics of the reaction rather than of the ionization constants of the reactants. This behavior has been recognized in a number of reactions, particularly those involving carbonyl groups such as Schiff base formation and hydrolysis (18–20) and condensation of formaldehyde with tetrahydrofolic acid (21) and other tetrahydroquinoxalines (22), and may be described as a change in the rate-determining step of the reaction with changing pH.

A change in the rate-determining step implies the presence of at least two steps and one intermediate. No intermediate was isolated or observed by spectroscopic, chromatographic, or polarographic methods. However, it is reasonable to postulate the presence of an imine or a more reactive species, the cationic imine, $R—\overset{+}{N}(—H)=CH_2$, which upon hydration gives the hydroxymethylamine, $R—NH—CH_2OH$. Decomposition of this carbinolamine then yields formaldehyde and disulfonamide or the appropriate aldehyde and sulfonamide.

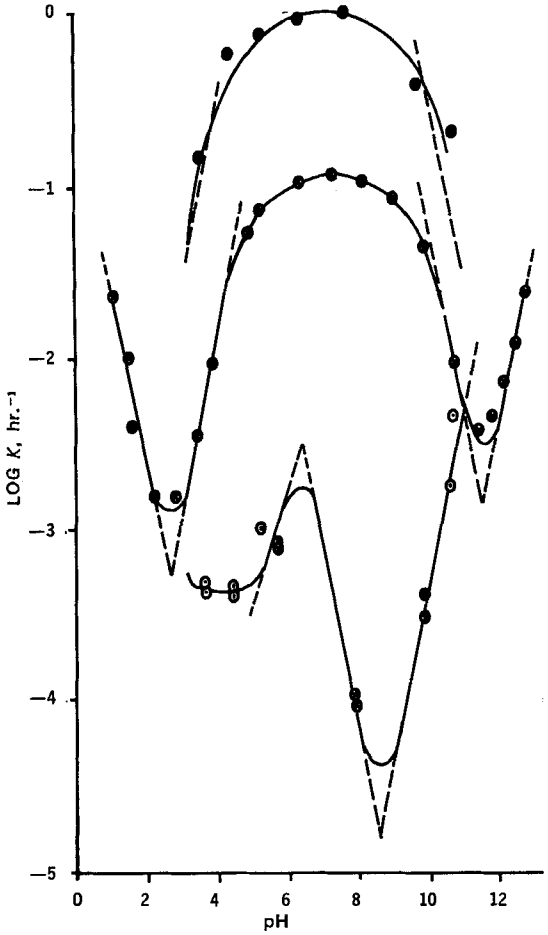


Figure 6—*The pH–rate profiles for the hydrolysis of* C*-ethyl hydrochlorothiazide, hydrochlorothiazide, and* N*-ethyl hydrochlorothiazide at 60° (top to bottom). Dashed lines are drawn with slope of +1 or −1.*

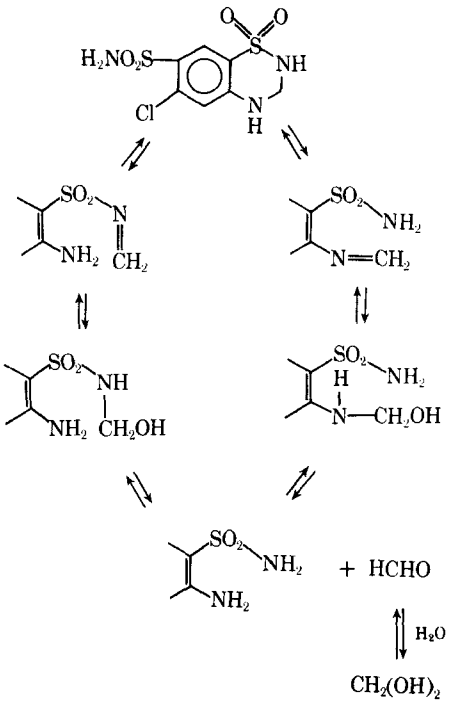
This type of scheme has been presented to account for the observed pH–rate profiles of other carbonyl group reactions and appears reasonable in this case also. Although there is no direct evidence of an intermediate in this case, formamide intermediates were reported in the hydrolysis of chlorothiazide (2), and analogous compounds were reported from the reaction of *o*-aminosulfonamides with various aldehydes, formic acid, and *ortho*-esters (23–25).

The pH–rate profile, the lack of significant buffer catalysis, the reports that aldehydes react with both the aniline nitrogen and the sulfonamide nitrogen, and the other evidence cited here all support the overall reaction scheme shown in Scheme I.

Table III—Effect of Various Buffers and Concentration on Extent and Rate of Reaction[a]

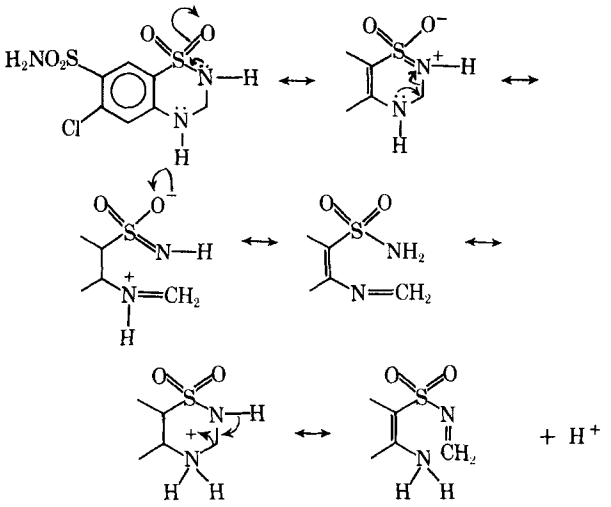
Compound	pH	Buffer, *M*	10^3k, hr.$^{-1}$	*Xe*
Hydrochlorothiazide	4.49	Acetate 0.12	2.76	0.42
	4.49	Acetate 0.24	2.74	0.41
	4.49	Acetate 0.36	2.76	0.41
	4.49	Acetate 0.48	2.79	0.41
C-Ethyl hydrochlorothiazide	5.20	Imidazole 0.10	59.8	0.80
	5.20	Imidazole 1.0	61.8	0.78
	7.75	Imidazole 0.10	87.1	0.81
	7.75	Imidazole 1.0	98.1	0.78
Hydrochlorothiazide	5.19	Imidazole 1.0	5.66	0.54
	5.31	Imidazole 0.10	6.27	0.43
	8.35	Imidazole 0.10	78.0	0.47
	8.35	Imidazole 1.0	77.9	0.70
C-Ethyl hydrochlorothiazide	10.78	Phosphate 0.05	20.4	0.81
	10.80	Phosphate 0.50	19.2	0.78

[a] $T = 60°$; hydrochlorothiazide $C_0 = 6.7 \times 10^{-4}$ *M*; *C*-ethyl hydrochlorothiazide $C_0 = 6.1 \times 10^{-4}$ *M*.



Scheme I

It can be shown that ring opening can occur *via* several pathways. Two examples are given in Scheme II. Either pathway yields an



Scheme II

imine intermediate and could account for the observed behavior.

The *N*-ethyl substitution in Su 6835 undoubtedly accounts for its relative unreactivity, being several orders of magnitude less reactive than hydrochlorothiazide or *C*-ethyl hydrochlorothiazide, since the replacement of the acidic proton suppresses ring opening. The electron-donating effect of the ethyl substitution at the 3-position, however, does accelerate the overall rate relative to hydrochlorothiazide; the observed effects of ethyl substitution in the 2- and 3-positions are, therefore, in accord with what one would predict and support the postulated mechanism.

It is postulated, therefore, that the overall reaction occurs as shown in Scheme I. The rate-determining steps could vary from acid/base-catalyzed ring opening through hydration of the free or cationic imine by water and/or hydroxide ion to the decomposition of the carbinolamine to yield disulfonamide and aldehyde.

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ACKNOWLEDGMENTS AND ADDRESSES

Received March 9, 1971, from the *Development and Control Department, CIBA Pharmaceutical Co., Summit, NJ 07901*

Accepted for publication May 24, 1971.

The technical assistance of Miss M. Schmid and Miss S. Seymour is gratefully acknowledged.