colored form should be maintained to be similar with those in the original form.

Tautomeric Switch of the Photochromic Osazone. Given the crystal structure and the photochromic characteristics above, a currently proposed mechanism for IId is a proton transfer in the chelate ring. The tautomer formed with the proton transfer upon light irradiation, formally, has an azo–phenyl chromophore which is not formulated in the original form (Scheme II). The hypsochromic shift of the colored form relative to the original osazone could be interpreted as being due to a conjugated phenylazo system of the osazone, as has been similarly discussed from the spectra of a related osazone group of dehydro-L-ascorbic acid phenylosazone.[11] The aldehyde and the allyl (or alkyl) groups are reported to be essential for the manifestation of photochromism of the osazone derivatives;[5] those groups may be playing a vital role in stabilizing the colored tautomer. It is possibly a new mechanism for a chelate hydrogen acting as a simple "switch" of the photochromic cycles. We expect that the rediscovered class of photochromic osazones will be subjected to further study because of the simplicity of the mechanism and the potential usefulness in various applications.

Acknowledgment. We are greatly indebted to Professor W. R. Scheidt (Notre Dame) for helpful advice on some aspects of this paper.

Registry No. IId, 132127-03-8.

Supplementary Material Available: Figure S1 displaying the atom labeling scheme and tables of atomic coordinates, anisotropic temperature factors for non-hydrogen atoms, and individual bond lengths and angles (6 pages). Ordering information is given on any current masthead page.

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Mechanism of Thiazolidine Hydrolysis. Ring Opening and Hydrolysis of 1,3-Thiazolidine Derivatives of *p*-(Dimethylamino)cinnamaldehyde

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Abstract: The hydrolysis reactions of 2-(*p*-(dimethylamino)styryl)-1,3-thiazolidine and the corresponding *N*-butyl and *N*-phenyl derivatives in the pH range 1–12 proceed via the iminium ion intermediate formed in an equilibrium ring-opening reaction. Such an intermediate was detected spectrophotometrically (λ_{max} = 480–525 nm). The fast formation of the iminium ion in ring opening of the *N*-phenyl-1,3-thiazolidine could be monitored at pH 3–10. Ring opening involves a pH-independent reaction at pH >4, which proceeds 2.25-fold slower in D_2O than in H_2O, and hydronium ion catalysis at low pH. General acid catalysis in ring opening was observed with acetic acid buffers. Ring opening of the *N*-butylthiazolidine occurs 250-fold more rapidly than with the *N*-phenyl derivative. The plot of log k_0 vs pH for aldehyde formation from the *N*-butyl-substituted thiazolidine has five unit changes in slope. The hydrolysis reactions subsequent to ring opening proceed with (a) attack of OH^- on the zwitterion (ionized thiol group) at high pH, (b) attack of OH^- on the positively charged species (un-ionized thiol group) at pH <10 (or the kinetically equivalent attack of water on the zwitterion), (c) attack of water on the positively charged species at pH <5, and (d) attack of water on the protonated dipositive species at low pH. There is an apparent p*K* of 6.3 in the hydrolysis reactions (aldehyde formation) of the *N*-butyl-substituted thiazolidine, which is a complex constant governing the reversible ring opening and protonation. The stability of the iminium ion intermediate has great influence on the shape of the pH–rate constant profiles and the interpretation of the apparent p*K* values. The hydrolysis of 2-(*p*-(dimethylamino)phenyl)-*N*-acetyl-1,3-thiazolidine at 90 °C is pH independent from pH 1–4 and hydronium ion catalyzed at pH >4. The reaction involves rate-determining ring opening, which is due to the poor stabilization of the developing carbonium ion when there is an *N*-acetyl substituent.

The hydrolysis reactions of acetal analogues in which oxygen has been replaced by nitrogen or sulfur have been actively studied in our laboratory[1–10] and others,[11–17] because such reactions can have great utility in shedding light on the mechanisms of specific and general acid catalyzed processes. Also compounds of these general types have important biochemical counterparts. We have previously investigated the reactions of cyclic acetals and acetal analogues having a 5-membered ring such as 2-substituted 1,3-dioxolanes,[10,18–22] 1,3-oxathiolanes,[1,10] 1,3-oxazolidines,[4,5] and 1,3-imidazolidines,[6–9] i.e., O,O, O,S, O,N, and N,N derivatives.

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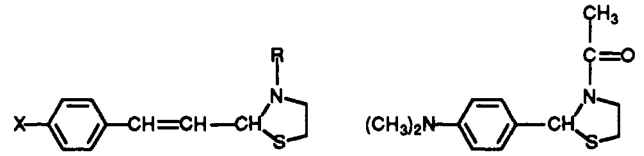
In the studies of the nitrogen analogues the reactions of derivatives of *p*-(dimethylamino)cinnamaldehyde have been particularly important[5,8,9] because it has often been possible to follow ring opening by observing the formation of an iminium ion intermediate. This has been possible because of the intense absorbance of the intermediate in the visible portion of the spectrum and the stability of the iminium ion to hydrolysis.

Thiazolidines, compounds containing N and S in a 5-membered ring, are of great interest because of the presence of a thiazolidine ring in the important antibiotic penicillin. Although there has been considerable mechanistic work on various aspects of the chemistry of penicillin,[23-26] the mechanism of thiazolidine ring opening has not been investigated, nor has it been established whether the reaction involves C–N or C–S bond breaking at moderately acidic or neutral pH values with either penicillin derivatives or simpler thiazolidines. We have, therefore, investigated the hydrolysis reactions of compounds I–V. Compound V has an amide nitrogen in the thiazolidine ring analogous to penicillin. Ring opening could be directly observed with II and III.



V

I: X = $N(CH_3)_2$; R = H
II: X = $N(CH_3)_2$; R = $-CH_2CH_2CH_2CH_3$
III: X = $N(CH_3)_2$; R = $-C_6H_5$
IV: X = H; R = $-CH_2CH_2CH_2CH_3$

Experimental Section

Materials. The thiazolidines I–IV were prepared by refluxing equivalent amounts of freshly sublimed *p*-(dimethylamino)cinnamaldehyde or cinnamaldehyde with β-aminoethanethiol, β-(butylamino)ethanethiol, or β-(phenylamino)ethanethiol and one drop of concentrated HCl in dry benzene. Water was continuously distilled from the reaction mixture by employing a Dean-Stark trap. A theoretical amount of water was collected. The mixture was cooled, neutralized with Na_2CO_3, and filtered. The benzene was then removed by rotary evaporation, and the residue was recrystallized.

After recrystallization from hexane 2-(*p*-(dimethylamino)styryl)-1,3-thiazolidine (I) melted at 76–77 °C. Anal. Calcd for $C_{13}H_{18}N_2S$: N, 11.96. Found: N, 11.99.

2-(*p*-(Dimethylamino)styryl)-*N*-butyl-1,3-thiazolidine (II) was recrystallized from hexane and melted at 48–49 °C. Anal. Calcd for $C_{17}H_{26}N_2S$: C, 70.34; H, 8.96; N, 9.66. Found: C, 69.98; H, 8.63, N, 9.57.

β-(Phenylamino)ethanethiol was prepared by the method of Reynolds.[27] 2-(*p*-(Dimethylamino)styryl)-*N*-phenyl-1,3-thiazolidine (III) was recrystallized from hexane and melted at 126 °C. Anal. Calcd for $C_{19}H_{22}N_2S$: C, 73.52; H, 7.15; N, 9.03. Found: C, 73.56; H, 7.09; N, 9.11.

2-Styryl-*N*-butyl-1,3-thiazolidine (IV) was hygroscopic and could not be obtained crystalline or distilled. Repeated precipitation from an ether–hexane mixture gave a sample with which there was no absorbance in the infrared spectrum that could be attributed to a carbonyl group. Anal. Calcd for $C_{15}H_{21}NS$: C, 72.84; H, 8.56; N, 5.67. Found: C, 72.49; H, 8.58; N, 5.63. A hydrochloride salt was prepared which melted at 254 °C dec.

2-(*p*-(Dimethylamino)phenyl)-1,3-thiazolidine was recrystallized from cyclohexane and melted at 137–138 °C. Anal. Calcd for $C_{11}H_{16}N_2S$: C, 63.46; H, 7.69; N, 13.46. Found: C, 63.58; H, 7.66; N, 13.34. 2-(*p*-(Dimethylamino)phenyl)-*N*-acetyl-1,3-thiazolidine (V) was prepared by acetylation of 2-(*p*-(dimethylamino)phenyl)-1,3-thiazolidine with excess acetic anhydride. After the mixture was stirred for 2 h, the excess acetic anhydride was removed by rotary evaporation. The residue was taken up in hot cyclohexane and filtered. Crystallization occurred upon allowing the mixture to stand at room temperature. After recrystallization from cyclohexane the white crystals melted at 68 °C. Anal. Calcd for $C_{13}H_{18}N_2OS$: C, 62.40; H, 7.20; N, 11.20. Found: C, 61.95; H, 7.03; N, 10.85.

Kinetic Measurements. The rates of hydrolysis of compounds I–V were measured spectrophotometrically with a Beckman Model 25 or a Pye-Unicam SP 8-100 recording spectrophotometer by following the absorbance increase due to appearance of aldehyde at 400 (I–III), 300 (IV), or 360 nm (V) and with I–III by monitoring the absorbance decrease at 480 or 525 nm. At pH <2 reactions of I–III were followed by monitoring the appearance of the protonated aldehyde at 280 nm. The ionic molarity of all buffers was maintained constant at 0.5 M with KCl. Stock solutions of substrate were prepared in anhydrous acetonitrile. Kinetic runs were initiated by injecting 15 μL of the substrate stock solution into 3 mL of temperature-equilibrated buffer in the cuvette. Reactions that were too rapid to be monitored with a conventional spectrophotometer were followed with use of a Durrum Model D-110 stopped-flow spectrophotometer. In rate measurements carried out with the stopped-flow spectrophotometer 150 μL of stock solution was mixed in one syringe with 15 mL of 0.5 M KCl solution buffered at pH ~8.0. The other syringe contained the appropriate buffer also with μ = 0.5 M. The reactions were pseudo-first-order for at least 4 half-lives. The values of k_{obsd}, the pseudo-first-order rate constant, were calculated with an IBM-370 computer. Reaction mixture pH values were measured with a Beckman 3500 digital pH meter. Second-order rate constants for hydroxide ion catalysis (k_{OH}) were calculated by using K_W values of 5.5 × 10^{-14} at 50 °C and 2.5 × 10^{-13} at 80 °C.

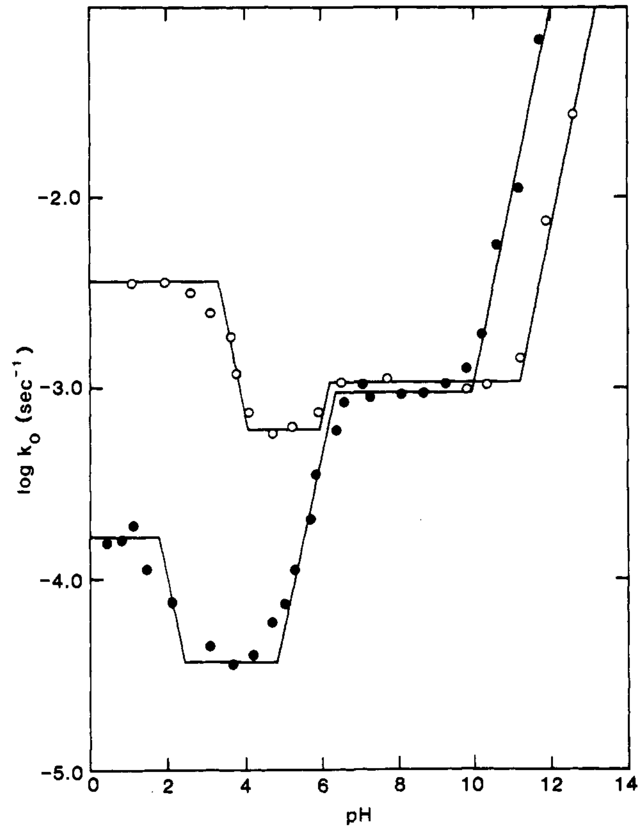


Figure 1. Plots of log k_0 vs pH for appearance of *p*-(dimethylamino)cinnamaldehyde or disappearance of the intermediates from 2-(*p*-(dimethylamino)styryl)-1,3-thiazolidine (O) and 2-(*p*-(dimethylamino)styryl)-*N*-butyl-1,3-thiazolidine (●) in H_2O at 50 °C and μ = 0.5 M with KCl.

Results

In Figure 1 are shown the plots of log k_0 vs pH for appearance of *p*-(dimethylamino)cinnamaldehyde from 2-(*p*-(dimethylamino)styryl)-1,3-thiazolidine (I) and 2-(*p*-(dimethylamino)styryl)-*N*-butyl-1,3-thiazolidine (II) in H_2O at 50 °C (μ = 0.5 M with KCl). The rate constants k_0 were obtained by extrapolation of k_{obsd} values to zero buffer concentration. These profiles represent rate-determining hydrolysis of an iminium ion (Schiff

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Table I. Rate Constants for Hydrolysis of 2-Substituted 1,3-Thiazolidines in H_2O at 50 °C (μ = 0.5 M with KCl)

compd	k_1, s^{-1}	k_2, s^{-1}	k_{OH}', M^{-1} s^{-1}	k_{OH}'', M^{-1} s^{-1}	$pK_{app,1}$	$pK_{app,2}$
I	3.6×10^{-3}	6.0×10^{-4}	13000	0.12	3.0	6.2
II	1.7×10^{-4}	3.5×10^{-5}	9100	2.0	1.8	6.3
III		7.6×10^{-4}	170	0.014		7.1
IV				0.057	5.0	
				0.36[a]	4.5	
V[b]	1.0×10^{-3}[c]				4.5	

[a] At 80 °C. [b] At 90 °C. [c] $k_1 = k_H K_a$.

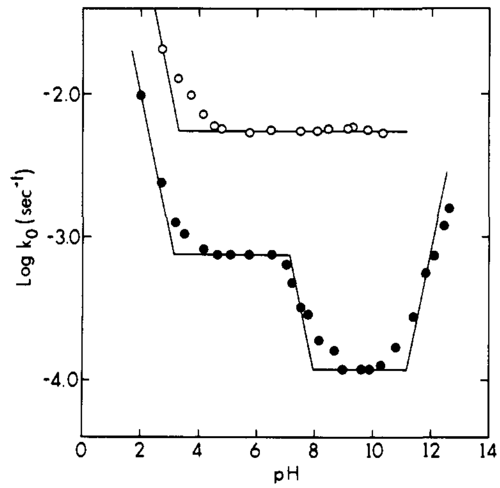

Figure 2. A plot of log k_{obsd} vs pH for ring opening of 2-(*p*-(dimethylamino)styryl)-*N*-phenyl-1,3-thiazolidine to the corresponding iminium ion at 30 °C (O) and a plot of log k_0 vs pH for hydrolysis of the iminium ion at 50 °C (●) in H_2O and μ = 0.5 M with KCl.

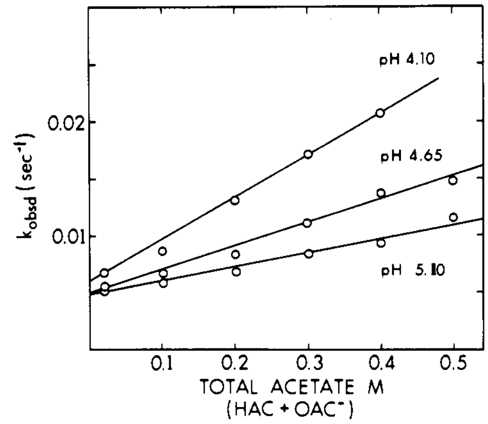

Figure 3. Plot of k_{obsd} vs the total concentration of acetic acid buffers for the ring opening of 2-(*p*-(dimethylamino)styryl)-*N*-phenyl-1,3-thiazolidine to the corresponding iminium ion at 30 °C in H_2O and μ = 0.5 M with KCl.

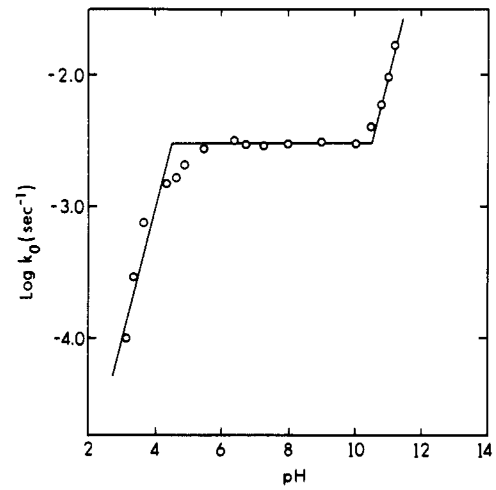

Figure 4. Plot of log k_0 vs pH for appearance of cinnamaldehyde from 2-styryl-*N*-butyl-1,3-thiazolidine in H_2O at 80 °C and μ = 0.5 M with KCl.

base) intermediate produced in a rapid ring-opening reaction. The intermediate derived from I and II could be observed spectrophotometrically at pH <9 and had λ_{max} = 480 nm. The observed rate constants in the plots of Figure 1 were identical at pH <9 for Schiff base disappearance measured by the absorbance decrease at 480 nm and aldehyde formation measured by the absorbance increase at 400 nm. The absorbance at 480 nm due to the intermediate from II declines with increasing pH after pH 6.3 and can no longer be detected at pH >9. Therefore, at pH values greater than 9 the reactions could only be followed by monitoring the appearance of aldehyde at 400 nm. With both I and II there is a OH^--catalyzed reaction at pH >9. At pH <9 the hydrolysis reactions are pH independent to pH values near 6. In the hydrolysis of the *N*-butyl-substituted thiazolidine II the plot of log k_0 vs pH bends downward near pH 6 (pK_{app} = 6.3) and then becomes pH independent at pH 5. At pH <4 an apparent hydronium ion catalyzed reaction takes place. The reaction again becomes pH independent near pH 2. The plots of Figure 1 have been drawn with theoretical unit slopes of 1.0, 0, and −1.0 to illustrate clearly the changes in slope and the pK_{app} values. The profile for hydrolysis of I is similar in shape to that of II, although the reactions of I are considerably faster at low pH. The rate constants determined from the plots of Figure 1 are given in Table I.

By utilizing stopped-flow rate measurements at 30 °C, the ring-opening reaction of the *N*-butyl derivative II could be followed by the initial absorbance increase at 480 nm in the pH range 3.5–6.5. The experimental values of k_{obsd} provide a good fit to eq 1, where k_1' is the rate constant for pH-independent breakdown

$$k_{obsd} = [k_1' + k_H a_H]\left[\frac{K_a}{K_a + a_H}\right] \qquad (1)$$

of II to an iminium ion and k_H is the second-order rate constant for the hydronium ion catalyzed ring opening. The values of the constants (30 °C) were taken to be $k_1' = 1.4$ s^{-1}, $k_H = 7 \times 10^5$ M^{-1} s^{-1}, and $K_a = 3 \times 10^{-5}$ M. At pH values less than 3.5 and greater than 6.5 the absorbance changes were not sufficiently large to accurately determine the rate constants for ring opening.

Figure 2 shows a plot of log k_{obsd} vs pH at 30 °C for ring opening of 2-(*p*-(dimethylamino)styryl)-*N*-phenyl-1,3-thiazolidine (III), which was monitored by following the absorbance increase at 525 nm. At pH >4 the reaction is pH independent ($k_1' = 5.7 \times 10^{-3}$ s^{-1}). The reaction is slower in D_2O than in H_2O; at pD 6.15 and 6.65 (cacodylate buffer) k_{obsd} (30 °C) is 2.51×10^{-3} and 2.55×10^{-3} s^{-1}, respectively ($k_{H_2O}/k_{D_2O} = 2.25$). Hydronium ion catalysis is observed at pH <4. There is only a slight inflection in the profile near pH 3.5 indicative of the pK_a of the *p*-(dimethylamino) group conjugate acid. The reaction at lower pH must be hydronium ion catalyzed ring opening of the protonated species. General acid catalysis in the ring-opening reaction by acetic acid buffers was clearly observed as shown in Figure 3. The value of the second-order rate constant k_{HA} is 0.046 M^{-1} s^{-1}.

Also included in Figure 2 is the plot of log k_0 (at zero buffer) vs pH for hydrolysis of the iminium ion produced from III at 50 °C (μ = 0.5 M). As with I and II there is hydroxide ion catalysis at high pH. The sigmoidal portion of the profile has an apparent pK_a of 7.1. Rate constants are given in Table I.

The plot of log k_0 vs pH for hydrolysis of 2-styryl-*N*-butyl-1,3-thiazolidine (IV) at 80 °C is shown in Figure 4. Again hydroxide ion catalysis is observed. There is an apparent pK_a at pH 4.5. At lower pH the rate of the reaction declines with decreasing pH. The apparent pK_a is 5.0 at 50 °C. Spectrophotometric titration at 30 °C and 295 nm revealed a pK_a of 4.6.

A plot of log k_{obsd} vs pH for hydrolysis of 2-(*p*-(dimethylamino)phenyl)-*N*-acetyl-1,3-thiazolidine (V) at 90 °C and μ =

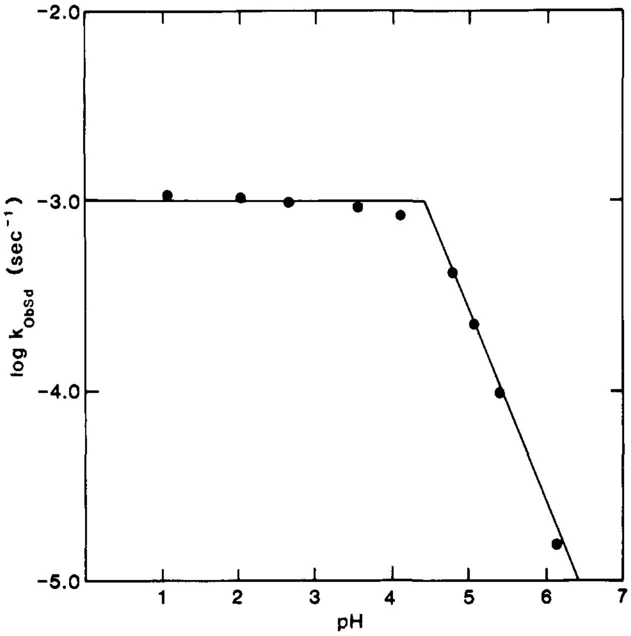

Figure 5. Plot of log k_{obsd} vs pH for hydrolysis of 2-(*p*-(dimethylamino)phenyl)-*N*-acetyl-1,3-thiazolidine in H_2O at 90 °C and μ = 0.5 M with KCl.

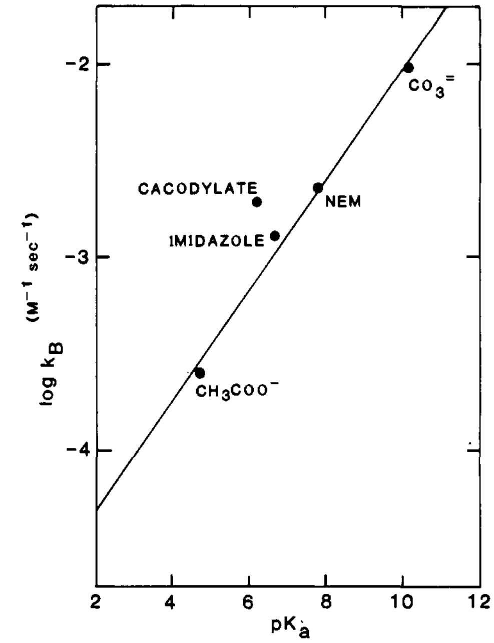

Figure 6. Plot of log k_B vs the pK_a of the conjugate acid of the general base catalyst in the hydrolysis of 2-(*p*-(dimethylamino)styryl)-*N*-butyl-1,3-thiazolidine in H_2O at 50 °C and μ = 0.5 M with KCl.

Table II. Second-Order Rate Constants for General Base Catalyzed Hydrolysis of 2-(*p*-(Dimethylamino)styryl)-1,3-thiazolidine and 2-(*p*-(Dimethylamino)styryl)-*N*-butyl-1,3-thiazolidine in H_2O at 50 °C (μ = 0.5 M with KCl)

compd	buffer	pK_a	10^3k_B, M^{-1} s^{-1}
I	acetate	4.70	5.6
	cacodylate	6.20	23
	phosphate	7.05	50
II	acetate	4.70	0.25
	cacodylate	6.20	1.9
	imidazole	6.80	1.25
	N-ethylmorpholine	7.80	2.30
	carbonate	10.20	9.8

0.5 M is presented in Figure 5. The reaction is pH independent in the pH range 1–4 with k_{obsd} = 0.001 s^{-1}. At pH >4 the slope of the plot of Figure 5 is −1.0. The equation for k_{obsd} is given in eq 2, where K_a is the dissociation constant of the conjugate acid; k_H at 90 °C is 26 M^{-1} s^{-1}, and pK_a is 4.5.

$$k_{obsd} = \frac{k_H a_H K_a}{K_a + a_H} \qquad (2)$$

Pronounced buffer catalysis was observed in the hydrolysis (aldehyde formation) of the thiazolidines (I–IV) but not in the hydrolysis of the *N*-acetyl derivative V. Studies at several constant pH values showed that the observed catalytic effect of the buffer in the hydrolysis of I–IV was due to the base species. Second-order rate constants k_B for general base catalyzed hydrolysis of I and II are given in Table II. The Bronsted plot of log k_B vs the pK_a of the conjugate acids of the base catalysts for general base catalyzed hydrolysis of II is shown in Figure 6. The slope of this plot is 0.29.

Discussion

A cationic Schiff base intermediate can be observed spectrophotometrically in the hydrolysis of 2-substituted 1,3-oxazolidines[4,5] and imidazolidines.[6-9] With derivatives of *p*-(dimethylamino)cinnamaldehyde the intermediate was detected by its intense absorbance at wavelengths in the visible at 480 nm or higher. Therefore, it is reasonable that a Schiff base might also be formed in the ring opening of thiazolidines if a thiol is a sufficiently good leaving group. Protonation of the ring would occur more readily on nitrogen than sulfur, but the most stable intermediate would result from C–S bond breaking rather than C–N. Schiff bases have been suggested as possible intermediates in thiazolidine hydrolysis in strongly alkaline solution.[28,29] However, such intermediates have not been clearly observed, and there has been little previous evidence as to the manner of hydrolysis of thiazolidines as a function of pH (a sulfonium ion intermediate was suggested in acidic solutions).[28] A Schiff base intermediate with λ_{max} = 480 nm (I and II) or 525 nm (III) has now been directly observed spectrophotometrically in the reactions of the *p*-(dimethylamino)cinnamaldehyde derivatives I–III at pH less than 10,[30,31] and the values of k_{obsd} for the hydrolysis reaction are the same when disappearance of Schiff base or appearance of aldehyde is followed at 400 nm. The fast ring-opening reactions of II and III could be followed by monitoring the initial absorbance increase at 480 or 525 nm, respectively, in the pH range 2.7–10. At the conclusion of the ring-opening reaction there was then a much slower decline in absorbance at 480 or 525 nm due to hydrolysis of the intermediate. The OH^--catalyzed reactions of the N-substituted thiazolidines at pH >10 (aldehyde formation) are, of course, only explainable in terms of a reaction of the Schiff base intermediate.

Ring Opening. Figure 2 shows the pH dependence of k_{obsd} for the approach to equilibrium in the ring opening of III. The plot must primarily represent the effect of pH on the forward reaction. A pH-independent reaction occurs in the pH range 4–10. A unimolecular or water-promoted decomposition reaction of the neutral species VI would be pH independent at pH values above the pK_a of the monocation. A similar pH–rate constant profile for ring opening was found in the reaction of II by utilizing stopped-flow measurements over the limited pH range 3.5–6.5. The pH-independent reaction of II is 250-fold more rapid than that of III, in accord with the greater ease of electron release from the alkyl-substituted nitrogen in the ring-opening process. Carbon–sulfur bond-breaking reactions that are pH independent also

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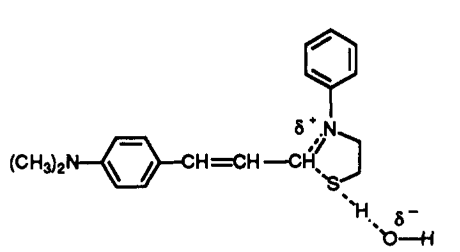
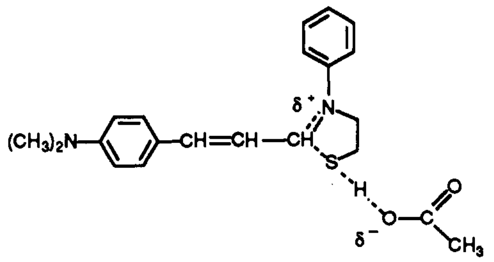
(30) A λ_{max} of 480 or 525 nm is in accord with the values found for other Schiff base derivatives of *p*-(dimethylamino)cinnamaldehyde.[5,8,9]
(31) Spectral evidence has also been obtained for a Schiff base in reactions of 2-(*p*-methoxyphenyl)-1,3-thiazolidine in HCl solutions. Fife, T. H.; Hutchins, J. E. C. Unpublished data. See ref 6.



VI

occur in the hydrolysis of acyclic *O,S*-thioacetals.[2] In those cases, the reactions proceed at nearly the same rate in D_2O as in H_2O and are, therefore, unimolecular decompositions. In contrast, the pH-independent ring opening of III is 2.25-fold slower in D_2O than in H_2O, which indicates the involvement of water as a proton transfer agent (VI). Proton transfer to sulfur in the transition state would avoid an unstable zwitterion as a discrete intermediate at pH <10.

The ring-opening reaction of III to the iminium ion is general acid catalyzed as illustrated in VII. In cases where there are rapid



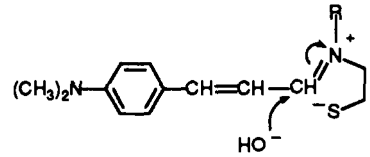
VII

hydronium ion catalyzed and pH-independent reactions, the most favorable pH to search for general acid catalysis is at the intersection point between those reactions.[32] Accordingly, with III, significant catalysis by acetic acid was observed at pH values close to 4.5 (Figure 3), but buffer catalysis was not observed at higher pH values, e.g., pH 8 with Tris buffers. As pH is increased the efficiency of general acid catalysis will decline (depending on the magnitude of α, the Bronsted coefficient) and will not compete with the pH-independent reaction.[32] General acid catalysis by buffer acids was not observed in the hydrolysis of *O,S*-thioacetals in cases where C–S bond breaking is rate determining,[2,17] even though pronounced general acid catalysis occurs in the hydrolysis of exactly analogous *O,O*-acetals. This was considered[2] to be due to the greater difficulty of unimolecular C–S bond breaking than C–O, as indicated by the 1000-fold less favorable unimolecular decomposition reactions of *S*-phenyl thioacetals than *O*-phenyl acetals when the leaving groups are of comparable basicity.[33,34] It was suggested[2] that general acid catalysis might be observed if the bond-breaking process could be made facile. In searching for such catalysis one cannot significantly improve the leaving group beyond *p*-nitrothiophenoxide because that increases greatly the magnitude of the rate constant for the pH-independent reaction, to the point that with dinitro substitution the reaction is pH independent even at pH ~1.[2] Weak but significant general acid catalysis can be detected in the intramolecular reactions of *S*-salicyl *O,S*-thioacetals.[3] It is now clear that general acid catalysis by buffer acids can be observed in reactions involving C–S bond breaking when the developing carbonium ion is highly stabilized in the transition state. It is undoubtedly this factor that is responsible for the acetic acid catalysis in the ring opening of III. As with acetals, general acid catalysis arises as a facilitation of the pH-independent decomposition reaction.[35]

A hydronium ion catalyzed reaction also takes place at low pH in the ring-opening reaction. The pK_a of the thiazolidine ring nitrogen and *p*-(dimethylamino) group conjugate acids of both II and III is 4.5 or less (see the discussion below). There is a small apparent inflection in the profile of Figure 2 for ring opening of III near pH 3.6. Likewise, the pK_{app} in ring opening of II is 4.5. Hydronium ion catalysis in the reaction of the neutral species or unimolecular decomposition of the monocation will be pH independent below the pK_a of the appropriate nitrogen conjugate acid.[10] At pH values below the pK_a of the monocation a unimolecular or water-promoted reaction of the neutral species would give a plot of log k_{obsd} vs pH with a slope of 1.0, and at pH values less than the pK_a of the diprotonated species the slope would be 2.0. Therefore, such a reaction would be very unfavorable at low pH. The ring-opening reaction could involve stepwise or concerted proton transfer to sulfur from an N-protonated species. The apparent hydronium ion catalyzed reaction at lower pH would then be due to formation of a dicationic species of $pK_a < 2$ or a kinetic equivalent, i.e., a hydronium ion catalyzed reaction of the monocation. The ring opening at low pH of 2-(*p*-(dimethylamino)styryl)-*N'*-phenyl-1,3-oxazolidine proceeds in that manner.[5] It should be noted that an apparent pK_a near 4 is not observed in the plots of log k_0 vs pH in Figures 1 and 2 for hydrolysis of II and III to the aldehyde.

Schubert and Motoyama[36] found that acyclic alkyl α-(dimethylamino)benzyl sulfides give *N,N*-dimethylbenzaliminium ion in aqueous acidic solution in a reaction involving cleavage of the C–S bond of the neutral species. *p*-(Dimethylamino)benzaldehyde *O*-(β-mercaptoethyl) *S*-(β-hydroxyethyl) thioacetal also undergoes a unimolecular decomposition with C–S bond breaking.[10] The reaction of the neutral species is favored, but the N-protonated species at pH <2 also reacts rapidly in a pH-independent process. Thus, there is ample precedence for unimolecular C–S bond-breaking reactions of acyclic derivatives, even when there is a proton in the molecule. However, at low pH proton transfer from nitrogen to sulfur could facilitate the reaction of the cyclic derivatives by (1) enhancing the ease of C–S bond breaking, (2) increasing the internal stabilization of the developing carbonium ion by nitrogen, and (3) removing the need for the formation of an unstable zwitterion in acidic solution. Thus, a mechanism involving proton transfer could provide a favorable pathway in ring-opening reactions. A diprotonated species would very likely require transfer of a proton to sulfur, since otherwise there could be little internal carbonium ion stabilization in the transition state.

Iminium Ion Hydrolysis. Since the measured rate constants for ring opening of II and III are much larger than those of iminium ion disappearance, the rate-determining step in the overall reaction must be hydrolysis of the iminium ion intermediate at all pH values. The plot of log k_0 vs pH for the OH^--catalyzed reaction of a zwitterionic Schiff base (VIII) will, of course, have a slope of 1.0.[6,37] At lower pH values (1–7) attack of water on cationic Schiff bases occurs and is pH independent.[6,37] The hy-



VIII

drolysis of the iminium ion intermediates from I–III is hydronium ion catalyzed at low pH. The catalysis must occur via protonation of the *p*-(dimethylamino) group, which will destabilize the iminium ion toward hydrolysis (IX). Such hydronium ion catalysis has

(32) Fife, T. H.; Anderson, E. *J. Org. Chem.* **1971**, *36*, 2357.

(33) The pH-independent unimolecular breakdown of benzaldehyde *O*-ethyl *S*-phenyl thioacetal is 10^3 slower than the corresponding reaction of benzaldehyde methyl *m*-nitrophenyl acetal.[17]

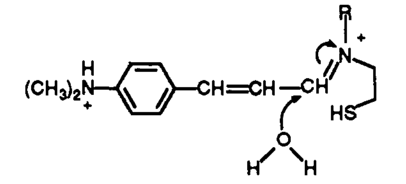
(34) The ElcB elimination of thiol anions from esters is 10^3 slower than that of oxygen anions of comparable pK_a. Pratt, R. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1970**, *92*, 5956.

(35) Fife, T. H. *Adv. Phys. Org. Chem.* **1975**, *11*, 1.

(36) Schubert, W. M.; Motoyama, Y. *J. Am. Chem. Soc.* **1965**, *87*, 5507. There is an inverse relationship between k_{obsd} and acid concentration for decomposition of α-(dimethylamino)benzyl ethyl sulfide in moderately concentrated acid. A unimolecular breakdown of the neutral species was suggested.

(37) Milakofsky, L. Ph.D. Thesis, University of Washington, 1967.

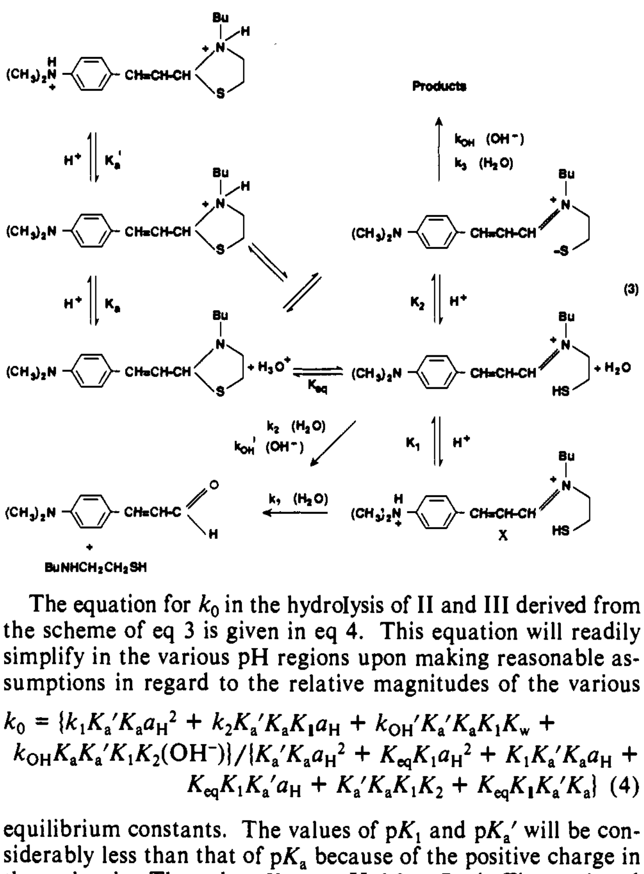
been observed previously in the hydrolysis of iminium ions derived



IX

from 1,3-imidazolidines and oxazolidines of p-(dimethylamino)cinnamaldehyde.[5,8,9] This reaction will become pH independent near the pK_a of the p-(dimethylamino) group (pK_1). Thus, the hydrolysis reactions of II and III at both low and high pH can be interpreted in a straightforward manner. However, the log k_0 vs pH profiles of Figures 1 and 2 in the pH range 3–10 include four changes in slope, which requires a detailed examination of the reaction as a function of pH.

The overall hydrolysis of the 2-substituted 1,3-thiazolidines in which an aldehyde is produced is depicted in eq 3, employing the N-butyl derivative II as an example, and considering the reaction at zero buffer concentration.



The equation for k_0 in the hydrolysis of II and III derived from the scheme of eq 3 is given in eq 4. This equation will readily simplify in the various pH regions upon making reasonable assumptions in regard to the relative magnitudes of the various

$$k_0 = \{k_1K_a'K_aa_H^2 + k_2K_a'K_aK_1a_H + k_{OH}'K_a'K_aK_1K_w + k_{OH}K_aK_a'K_1K_2(OH^-)\}/\{K_a'K_aa_H^2 + K_{eq}K_1a_H^2 + K_1K_a'K_aa_H + K_{eq}K_1K_a'a_H + K_a'K_aK_1K_2 + K_{eq}K_1K_a'K_a\} \quad (4)$$

equilibrium constants. The values of pK_1 and pK_a' will be considerably less than that of pK_a because of the positive charge in the molecule. Thus, the pK_{app} at pH 6.3 or 7.1 in Figures 1 and 2 must be a reflection of K_a, K_{eq} (a composite constant governing ring opening and protonation), or K_2. The change in slope at pH 6.3 or 7.1 cannot be due to pK_a since there will only be an inflection in the hydrolysis profile at pK_a if K_{eq} is larger than K_a; the absorbance at 480 or 525 nm due to the iminium ion at high pH shows that pK_{eq} cannot be less than 6.3. The absorbance at 480 nm due to the iminium ion from II declines as the pH is increased beyond pH 6.3, and at pH >8.5 the absorbance at 480 nm becomes negligible, which is consistent with pK_{eq} = 6.3 or greater. The iminium ion derived from III still displays absorbance at 525 nm at pH >10. The pK_a of the N-phenyl ring nitrogen conjugate acid of III must be considerably less than the pK_{app} at pH 7.1. Note also that there is no inflection at pH 7.1 in the log k_0 vs pH profile for ring opening of III. The pK_{app} of 4.5 in the ring opening of II is lower than might be expected for the pK_a of the ring nitrogen conjugate acid; nevertheless, a spectrophotometric titration of IV,

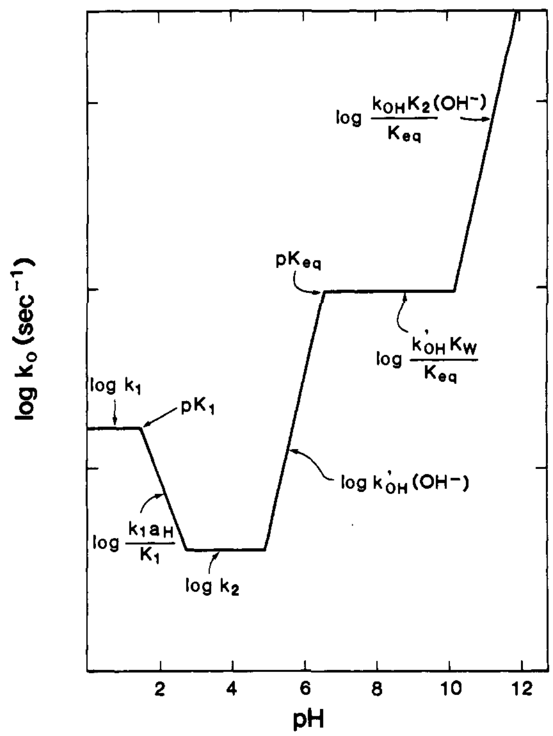


Figure 7. Plot of log k_0 vs pH for hydrolysis of 2-(p-(dimethylamino)-styryl)-N-butyl-1,3-thiazolidine in H_2O at 50 °C.

in which there is only one nitrogen in the molecule, indicated a pK_a of 4.6 at 30 °C.[38]

The profiles for I–III can be simply explained if it is assumed that the equilibrium constants are in the order $K_1 \sim K_a' > K_a > K_{eq} > K_2$. At low pH (<2) the pH-independent region in the profiles of I and II is a reflection of k_1 since $a_H > K_1$. Equation 5 holds at low pH if $K_a'K_a > K_1K_{eq}$, so that at $K_1 > a_H$ the apparent hydronium ion catalyzed reaction is due to the increased

$$k_0 = \frac{k_1a_H + k_2K_1}{a_H + K_1} \quad (5)$$

rate of hydrolysis of the iminium ion (X) when the p-(dimethylamino) group is protonated.[39] At higher pH ($K_1 > a_H$) the reaction becomes pH independent when k_2K_1 becomes larger than k_1a_H. At still higher pH an apparent OH^--catalyzed reaction is observed in the reaction of I and II, and k_0 is given by eq 6. The reaction will again be pH independent at $K_{eq} > a_H$. The

$$k_0 = \frac{k_2a_H + k_{OH}'K_w + k_{OH}(OH^-)K_2}{a_H + K_{eq}} \quad (6)$$

pH-independent reaction will then be given by eq 7, and the OH^--catalyzed reaction at higher pH by eq 8. Thus, the various regions and inflections of the profile of Figure 1 would have the values shown in Figure 7.[40]

$$k_0 = \{k_{OH}'K_w\}/K_{eq} \quad (7)$$

$$k_0 = \{k_{OH}(OH^-)K_2\}/K_{eq} \quad (8)$$

If the equilibrium constants were in the order $K_1 \sim K_a' > K_a > K_2 > K_{eq}$, then eq 9 would be obtained for k_0 at pH >4, and the profile would bend downward at pK_2. This possibility is not

(38) A low pK_a for the nitrogen conjugate acid might result from steric inhibition of solvation by the butyl group substituent.

(39) If, on the other hand, $K_1K_{eq} > K_a'K_a$, then the values of k_0 at low pH will be determined by complex constants, i.e., $k_1K_a'K_a/K_1K_{eq}$ and $K_{app,1} = K_a'K_a/K_{eq}$.

(40) A change in rate-determining step at pK_{app} can be ruled out in the hydrolysis of II and III. The description of the equilibrium ring-opening reaction in terms of K_{eq} makes no assumptions in regard to mechanism.

in accord with the log k_0 vs pH profile for hydrolysis of I, which

$$k_0 = \frac{k_2 a_H + k_{OH}' K_w + k_{OH}(OH^-) K_2}{a_H + K_2} \quad (9)$$

shows that $K_{eq} > K_2$ in that reaction. Also note that at high pH ($K_2 > a_H$), eq 10 would hold if eq 9 were being followed. The

$$k_0 = k_{OH}(OH^-) \quad (10)$$

experimental values of k_{OH}'' in Table I are, however, not reasonable for OH^- attack on a cationic Schiff base. For example, the k_{OH} value for hydrolysis of *p*-methoxybenzal *N,N*-dimethyliminium ion is 5×10^4 M^{-1} s^{-1} at 30 °C.[6] Thus, the values of k_{OH}'' in the hydrolysis of I–III (Table I) must be modified by the equilibrium ring-opening step (eq 8). The inflections in the log k_0 vs pH profiles cannot then be a reflection of K_2, i.e., K_{eq} must be greater than K_2. The k_{OH}' value in Table I for hydrolysis of II is, however, in accord with that expected for attack of OH^- on a cationic Schiff base as required by eq 6 at $a_H > K_{eq}$. Employing that value of k_{OH}' and eq 7, K_{eq} can be calculated to be 5.3×10^{-7} M, which is consistent with the value obtained from Figure 1. With eq 8 and a reasonable estimate of k_{OH} for hydrolysis of II, K_2 may then be calculated to be 1.2×10^{-10} M ($pK_2 = 9.9$). Thus, the data for hydrolysis of I–III is consistent with eq 6–8.

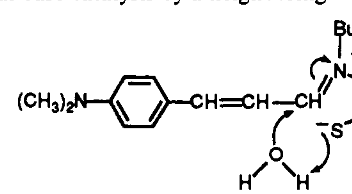
If K_{eq} is very large so that $K_{eq} > K_a > K_2$, then eq 4 simplifies to eq 11. The plot of log k_0 vs pH will then have inflections at

$$k_0 = \{k_1 K_a' K_a a_H^2 / K_1 + k_2 K_a' K_a a_H + k_{OH}' K_a' K_a K_w + k_{OH} K_a' K_a K_2 (OH^-)\} / \{K_{eq} [a_H^2 + K_a' a_H + K_a' K_a]\} \quad (11)$$

pK_a' and pK_a. Such a plot would be expected in the hydrolysis of thiazolidines giving a much less stable iminium ion than that derived from I–III. This situation is very likely realized in the hydrolysis of 2-styryl-*N*-butyl-1,3-thiazolidine (IV) with which the kinetically determined pK_{app} and the titrimetrically determined pK_a are in good accord. Thus, the interpretation of the observed rate constants for thiazolidine hydrolysis will vary depending upon the relative magnitudes of the equilibrium constants. Nevertheless, the reactions must be (a) attack of OH^- on the zwitterion (ionized thiol group) at high pH, (b) attack of OH^- on the positively charged species (un-ionized thiol group) at pH <10 (or the kinetically equivalent attack of H_2O on the zwitterion), (c) attack of water on the positively charged species, and (d) attack of water on the protonated dipositive species at low pH.

The shape of the pH–log rate constant profile for aldehyde formation and the interpretation of the pK_{app} values is critically dependent upon the stability of the intermediate iminium ion in this type of hydrolytic reaction involving an equilibrium ring-opening step. In the hydrolysis of the iminium ions derived from II and III the plots of log k_0 vs pH bend downward at pK_{eq} as required by eq 6, and that equation provides an excellent fit of the data points in the hydrolysis of both compounds. However, the general shapes of the two profiles in Figures 1 and 2 are reversed. An apparent hydroxide ion catalyzed reaction of the positively charged species (un-ionized thiol group) will only be reflected in the log k_0 vs pH profile if it is facile enough to occur at pH values less than pK_{eq} (at $a_H > K_{eq}$ in eq 6). The observation of an apparent OH^--catalyzed reaction at pH values below pK_{eq} will depend on the relative values of $k_{OH}'(OH^-)$ (or the kinetically equivalent rate constant for attack of water on the zwitterion (k_3)) and k_2. These rate constants will depend both on the ability of the N-substituted nitrogen to release electrons to stabilize the iminium ion and on the steric bulk of the N substituent. Apparent hydroxide ion catalysis is observed in the hydrolysis of II at low pH because water attack on the iminium ion (un-ionized thiol group) in the k_2 step is unfavorable. However, in the case of III, k_2 is 20-fold larger than with II, whereas the apparent OH^--catalyzed reaction governed by k_{OH}' is at least 50-fold less favorable than the comparable reaction of II.

Hydroxide ion attack on the iminium ion intermediates might reasonably be expected in the pH range 7–10, in accordance with the analogous reaction observed in the hydrolysis of *p*-methoxybenzal *N,N*-dimethyliminium ion at pH >7.[6,37] Such an OH^--catalyzed reaction would give a log k_0 vs pH profile with a slope of 1.0 at increasing pH values below the pK_{eq} of II and pH independence of log k_0 at higher pH in reactions of the cationic species. However, an OH^--catalyzed reaction at pH values as low as 5 (see Figure 1) might not be anticipated in the hydrolysis of iminium ions on the basis of previous investigations.[5-9,37] For example, the hydrolysis of substituted benzylidene-1,1-dimethylethylamines in the pH range 5–9 was considered to involve attack of water on the protonated imine.[41] If the hydrolysis reaction of II involved attack of water on the zwitterionic species (ionized thiol group), the profile would also be linear with a slope of 1.0 to pK_{eq} and pH independent at higher pH. The pronounced general base catalysis in the hydrolysis of I and II by a wide variety of buffer bases in the pH range 5–10 suggests a reaction with water in which there is proton transfer in the transition state. Bases such as *N*-ethylmorpholine would not act as nucleophiles in these reactions. The calculated value of the rate constant k_3 that would be required for attack of water on the zwitterion from II is 4 s^{-1} at 50 °C, which is quite large for that type of reaction. Note that k_2 for water attack on the cationic Schiff base from II (un-ionized thiol group) is only 4×10^{-5} s^{-1} at 50 °C, and the rate constant for attack of water on *p*-methoxybenzal *N,N*-dimethyliminium ion is only 9×10^{-3} s^{-1} at 30 °C.[6] A facile intramolecular general base catalysis by the neighboring thiol anion would, however, significantly increase the magnitude of the observed rate constants in water reactions of the zwitterions (XI). A similar intramolecular general base catalysis by a neighboring amine group may

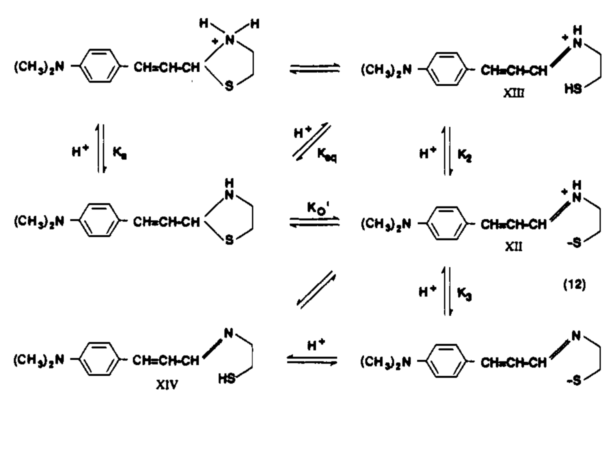


XI

occur in the hydrolysis of iminium ions derived from 1,3-imidazolidines.[6] Thus, the log k_0 vs pH profiles for hydrolysis of II and III are novel in form and may reflect at pH <10 reactions of water with the zwitterionic species, although there is no inflection in the plots of log k_0 vs pH at pK_2 because of the reversibility of the ring-opening reactions.

The scheme of eqs 3 and 4 also describes the reactions of the unalkylated thiazolidine I (Bu = H). Again, the observed rate constants will be a reflection of the reactivity of the iminium ion intermediate and the equilibrium concentration of the intermediate. The fast reactions of the unalkylated compound I at low pH relative to II can therefore be due to greater reactivity of the iminium ion produced in ring opening of I and/or a relatively greater concentration of that species. Both k_1 and k_2 are larger with I than II. A large alkyl substituent on nitrogen could sterically inhibit the attack of water on the iminium ion, but note that the k_0 values for I and II are quite similar in the pH range 6–10.

Proton transfer can occur in the ring opening of the unalkylated thiazolidine I or in the zwitterionic intermediate XII (eq 12). An



(12)

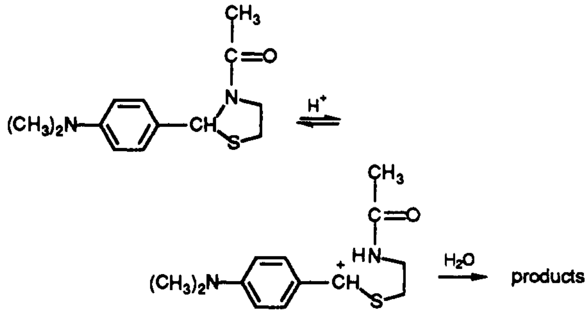
uncharged Schiff base (XIV) should be less susceptible to reclosure of the ring than a zwitterionic species. Water reactions of XII and XIV would be kinetically equivalent. However, in view of the similarity of the observed rate constants for I and II in the pH-independent reaction from pH 6 to 10, the reaction is very likely attack of OH^- on the cation XIII, or attack of water on the zwitterion XII, i.e., the N-alkylated derivative serves as a model for the N-protonated species XII and XIII. A neutral or anionic species is, of course, not possible in the reactions of the *N*-butyl derivative II.

The formation and hydrolysis of Schiff bases unsubstituted on nitrogen has been extensively studied.[41,42] At high pH values OH^- attack takes place on the protonated Schiff bases and is therefore pH independent. In contrast, the profile for hydrolysis of I shows that k_0 is still dependent on OH^- concentration at pH >11, which should be above the pK_3 of the zwitterionic Schiff base.[41] For this to occur, either ring opening must be rate determining (which is not the case with I), or the observed pH dependence of k_0 is influenced by the equilibrium ring-opening step. At high pH the plot of log k_0 vs pH will be linear with a slope of 1.0 in the hydrolysis of the zwitterionic species XII in eq 12 as long as $a_H > K_0'K_3$ with $K_0' < 1$, since k_0 will then be given by eq 13. When K_0' is less than unity K_{eq} must be greater than K_2. By employing

$$k_0 = \frac{k_{OH}K_0'K_w}{a_H + K_0'K_3} \qquad (13)$$

the relationship $K_{eq} = K_2/K_0'$ and the calculated values of K_{eq} and K_2 in the hydrolysis of II (5.3×10^{-7} and 1.2×10^{-10} M, respectively), K_0' may be calculated to be 2.3×10^{-4}. The unfavorable value of K_0' must be due to the rapid reclosure of the thiazolidine ring via attack of the thiol anion on the iminium ion. Ring opening will, therefore, only be favorable at pH values less than pK_2 so that the intermediate may be stabilized by protonation of the thiol group.

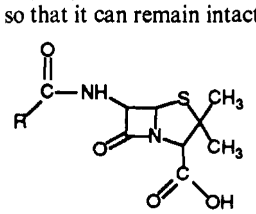
N-Acylated Thiazolidine Hydrolysis. The N-acylated derivative V hydrolyzes very slowly at 90 °C in comparison with the other compounds in the series. This is undoubtedly due to the electron-withdrawing effect of the *N*-acetyl group. Electron release from nitrogen in a ring-opening reaction involving C–S bond breaking would be hindered by the *N*-acetyl group, and as a result, stabilization of the developing carbonium ion would be significantly reduced. The hydronium ion catalyzed removal of the *N*-acetyl group is not the rate-determining step in the hydrolysis of V in view of the large sensitivity of k_{obsd} to the ionization state of the *p*-(dimethylamino) group (Figure 5), such that a hydronium ion catalyzed reaction of the protonated species is not observed at pH values as low as 1.0 (the pK_{app} of 4.5 is normal for the pK_a of the conjugate acid of the *p*-(dimethylamino) group).[10] Electronic effects are not significant in the hydronium ion catalyzed hydrolysis of amides,[43] e.g., the Hammett ρ value for hydrolysis of substituted benzamides is close to zero because of compensating effects of electron withdrawal on protonation and reaction of the conjugate acid with water. The hydrolysis of V must proceed with rate-determining ring opening or reaction of the intermediate with water. However, the lack of buffer catalysis is not consistent with rate-determining hydrolysis of a stabilized carbonium ion intermediate;[10] note that the hydrolysis of the iminium ions produced in ring opening of I–III is markedly catalyzed by buffer bases. Rate-determining ring opening of V is supported by the shape of the plot of log k_{obsd} vs pH in Figure 5. The profile for appearance of aldehyde is identical in shape with that for hydrolysis of *p*-(dimethylamino)benzaldehyde dipropyl acetal, which hydrolyzes by an A-1 mechanism.[10] In both cases the profile is pH independent from pH 1 to 4 and linear with a slope of −1.0 at pH >4. The pH-independent region in the profile for hydrolysis of the acetal reflects a transition from a hydronium ion catalyzed reaction of the neutral species to a slower hydronium ion catalyzed reaction of the protonated species (protonated *p*-(dimethylamino) group). Thus, at pH values from 1 to 6 the reaction of V involves rate-determining hydronium ion catalyzed ring opening of the neutral species, as illustrated in eq 14. A hydronium ion catalyzed ring



(14)

opening in the hydrolysis of V shows that when the thiazolidine nitrogen is substituted with a strongly electron withdrawing group, stabilization of the developing carbonium ion is then not sufficient to allow a unimolecular or water-promoted C–S bond-breaking reaction. The absence of buffer catalysis indicates that hydronium ion catalysis involves an equilibrium protonation step. Protonation of either nitrogen or sulfur with subsequent C–N or C–S bond breaking would lead to the release of *p*-(dimethylamino)benzaldehyde.[44] The amide function would be of greater basicity than sulfur. However, in either case, stabilization of the developing carbonium ion would be provided mainly by the *p*-(dimethylamino) group. Ring opening will only be rate determining if the carbonium ion intermediate reacts with H_2O faster than the ring recloses. Thus, nucleophilic attack by the amide nitrogen (eq 14) or the un-ionized sulfhydryl group does not compete with attack of 55 M H_2O.

Enzymatic and Nonenzymatic Reactions of Thiazolidine Derivatives. It is clear that the 1,3-thiazolidine ring will open rapidly at pH <10 when the developing carbonium ion is highly stabilized, and both pH-independent and hydronium ion catalyzed processes occur. There will normally be a high degree of carbonium ion stabilization if sulfur is the leaving group because the remaining nitrogen can release electrons readily to form the iminium ion intermediate. On the other hand, when an electron-withdrawing acyl function is substituted on the thiazolidine ring nitrogen, the ring-opening process is very slow and may be rate determining in the hydrolytic reaction. Consequently, the N-substituted amide function of penicillin (XV) will confer considerable stability on the thiazolidine ring so that it can remain intact prior to and during



XV

enzymatic reactions. However, the results with V show that the thiazolidine ring might still open in preference to C–N cleavage were it not for the high reactivity of the β-lactam ring. The thiazolidine ring is very likely important for steric reasons in the action of penicillin and may further increase the reactivity of the β-lactam carbonyl by increasing strain and/or by decreasing coplanarity and thereby reducing the resonance interaction between nitrogen and the carbonyl.[23,45–47] Opening of the thiazo-

(41) Cordes, E. H.; Jencks, W. P. *J. Am. Chem. Soc.* **1963**, *85*, 2843.

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lidine ring could then be important subsequent to the enzymatic acylation reaction.

The penicillins and cephalosporins are effective bacteriocidal agents presumably because they disrupt bacterial cell wall synthesis.[23,48,49] This effect is exerted by the inhibition of enzymes that catalyze the cross-linking reaction of peptidoglycan strands.[48,49] The process of transpeptidation is thought to involve the cleavage of the terminal D-alanyl-D-alanine of the peptidoglycan to give an acyl enzyme intermediate with release of D-alanine. Inhibition may arise because penicillin is a structural or transition-state analogue of acyl D-ala-D-ala.[50,51] It has been proposed that penicillin acylates the relevant transpeptidase enzymes via a nucleophilic reaction at the β-lactam carbonyl.[50-52] Removal in this manner of the amide substituent should allow the thiazolidine ring to open with reasonable rapidity. Indeed ring opening does occur in the breakdown of the benzyl penicillin–DD-carboxypeptidase–transpeptidase enzyme complex of Streptomyces R61.[52] Such a process could increase the difficulty of enzyme reactivation by altering the alignment of the acyl enzyme carbonyl to functional groups in the active site.

It has generally been considered that the product of alkaline hydrolysis of penicillin derivatives will be the corresponding thiazolidine, e.g., benzylpenicillin undergoes opening of the β-lactam ring to give benzylpenicilloic acid.[23,53] This implies that thiazolidine ring opening is unfavorable at those pH values. As seen in the present work the equilibrium for thiazolidine ring opening is indeed unfavorable at high pH (>10), even when the forward reaction is rapid because of significant internal stabilization of the developing carbonium ion. This is because the reverse ring-closure reaction involving nucleophilic attack of the thiol anion is facile. As a consequence, ring opening is only favorable at pH <10 where the thiol group is un-ionized. However, both the ring-opening and hydrolysis reactions are rapid and pH independent at pH values considerably greater than 7. Thus, the present studies of the hydrolysis of thiazolidines, in which conclusive evidence for an iminium ion intermediate was obtained with the derivatives of p-(dimethylamino)cinnamaldehyde, and in which a detailed kinetic analysis of the hydrolysis reactions was possible, have provided insight into the reactivity of the thiazolidine ring and into the kinetic consequences of preequilibrium ring-opening reactions. These factors may be important in the reactions of penicillin and its derivatives.

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Registry No. I, 130904-39-1; II, 130904-40-4; III, 130904-41-5; IV, 130904-42-6; V, 79661-90-8; p-(dimethylamino)cinnamaldehyde, 6203-18-5; cinnamaldehyde, 104-55-2; β-aminoethanethiol, 60-23-1; β-(butylamino)ethanethiol, 5842-00-2; β-(phenylamino)ethanethiol, 5977-99-1.

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Novel Method for Polysaccharide Synthesis Using an Enzyme: The First in Vitro Synthesis of Cellulose via a Nonbiosynthetic Path Utilizing Cellulase as Catalyst

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Abstract: The in vitro synthesis of cellulose via a nonbiosynthetic path has been achieved for the first time by condensation of β-D-cellobiosyl fluoride as substrate for cellulase, a hydrolysis enzyme of cellulose, in a mixed solvent of acetonitrile/acetate buffer (pH 5, 5:1). The water-insoluble part of the products is "synthetic cellulose", the structure of which was confirmed by comparison with an authentic natural cellulose sample with use of solid ^{13}C NMR and IR spectroscopies as well as with a hydrolysis experiment. The present synthetic cellulose was converted to the corresponding triacetate whose molecular weight was at least 6.3×10^3 (degree of polymerization (DP) ≥22). X-ray as well as ^{13}C NMR analyses showed that its crystal structure is of type II with high crystallinity. Under reaction conditions of a higher substrate concentration or higher acetonitrile concentration, water-soluble cellooligosaccharides (DP ≤ 8) were produced predominantly.

Cellulose is the most abundant organic substance occurring on the earth. Some 10^{15} kg of cellulose are photosynthesized and degraded each year.[1] For over a century, many researchers have been attracted by this natural substance, and enormous studies, structure determinations, biosyntheses, and chemical and physical properties determinations have been performed in view of both fundamental sciences and practical applications.[2] In vitro synthesis of cellulose, therefore, has long been one of the most difficult, yet important, challenging topics from the early stages of macromolecular science. Many efforts have been devoted to regio- and stereoselective preparation of cellulose, i.e., construction of stereoregular polysaccharides having $\beta(1\rightarrow4)$ glycosidic linkage. The chemical approaches so far attempted, however, have failed to solve the problem in spite of remarkable development of modern synthetic methods.[3,4] The condensation of 2,3,6-glucose tricar-

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