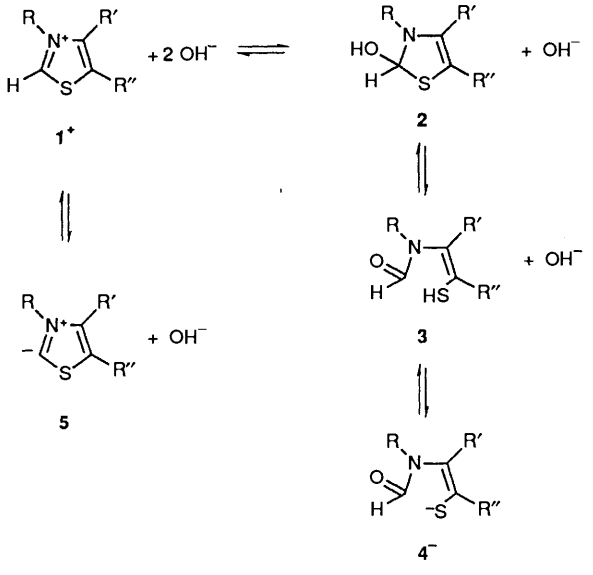
Reversible Hydrolysis of the 3-Benzyl-5-(2-hydroxyethyl)-4-methylthiazolium Cation in Aqueous Solution

Irmgard Heiber-Langer, Ingrid Winter and Wilhelm Knoche*
Fakultät für Chemie, Universität, D-4800 Bielefeld 1, Germany

The hydrolysis of 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide has been studied using a stopped-flow technique with spectrophotometric recording. This reaction consists of three steps: the binding of a hydroxide ion to the thiazolium cation to form the pseudobase, the ring-opening of the pseudobase, and the loss of a proton from the ring-opened form (Breslow mechanism). The pseudobase and ring-opened form are intermediates and cannot be observed at equilibrium. However, in the stopped-flow experiment the three steps can be observed separately, and all equilibrium and rate constants have been obtained. The pH dependence of the reaction rates indicates the existence of a further intermediate, which is formed by *N*-protonation of the pseudobase. The p*K* value of the catalytically active ylide is larger than 15, therefore its concentration is too small to be detected.

Thiazolium salts are versatile catalysts for many organic and biochemical reactions. The best known representative of these compounds is thiamine pyrophosphate (vitamin B_1), which acts as cofactor in some important enzymatic reactions.[1] According to Breslow,[2,3] the active catalytic species of thiazolium salts is the ylide, species **5** in Scheme 1, which is deprotonated at the



Scheme 1

C-2 atom. This ylide attacks positively polarized carbon atoms in carbonyl compounds, to form 'active aldehydes' which can react further with different substrates. The reaction of a thiazolium salt with base may also lead to the formation of other products. Breslow[4] summarized the results obtained for these reactions in Scheme 1, where a thiazolium salt $\mathbf{1}^+$ reacts *via* addition of OH^- to form the pseudobase **2**. Ring-opening is possible for this pseudobase, and the ring-opened thiol form **3** can be deprotonated to the anion $\mathbf{4}^-$. The ylide and the thiol form are of biological interest: the ylide, because it acts as a catalyst, and the thiol form, because, compared to thiamine, it is more easily transported through membranes *via* 'ring-opened disulfide derivatives'.[5]

The kinetics of the reactions involved have been studied by several authors,[5–18] but in no case could equilibrium and rate constants be determined for all steps in Scheme 1. On the other hand, for an optimal use of thiazolium salts as catalysts, these parameters should be known. Therefore we tried to exploit the possibilities of spectrophotometric and stopped-flow techniques in order to study the hydrolysis of a thiazolium cation as completely as possible.

We have chosen the 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium cation, because it is similar to thiamine but does not undergo the side reactions due to the pyrimidine moiety. For this compound forward and backward reactions were examined over the whole accessible pH range from 0 to 14.5.

Experimental

Materials.—3-Benzyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide (Tz) was prepared from 5-(2-hydroxyethyl)-4-methylthiazole and benzyl bromide.[19] It was recrystallized once from acetonitrile and then several times from a 1:1 mixture of acetone and ethanol. To the last mixture diethyl ether was added until turbidity could be observed in the solution. The crystals were collected by filtration and dried.

$NaHCO_3$, KH_2PO_4, $Na_2B_4O_{10}\cdot 10H_2O$ (Merck), citric acid (Riedel de Haen), tris(hydroxymethyl)aminomethane (Baker), NaOH (Titrisol Merck) or for more concentrated solutions NaOH (Merck) and HCl (Titrisol Merck) were used. All solutions were prepared with triply distilled water. The pH of the solutions was calculated from the concentrations of all compounds. The borate buffer solutions were prepared according to the data collected in Robinson and Stokes.[20] Activity corrections were calculated from the ionic strength I using eqn. (1).[21]

$$-\log f_i = 0.5\, z_i^2 \left(\frac{I^{0.5}}{1 + I^{0.5}} - 0.3\, I \right) \qquad (1)$$

Equilibrium Measurements.—The potentiometric titration was performed with a Beckmann 4500 digital pH meter and a Metrohm 655 dosimat. 10^{-3} mol of Tz was dissolved in 20 cm^3 of water and titrated with 1 mol dm^{-3} NaOH.

For the spectrophotometric measurements borate buffer solutions with different pH values containing 10^{-4} mol dm^{-3} Tz were prepared. Their absorbance was measured using a Cary Varian 219 spectrophotometer.

Kinetic Measurements.—For slow reaction rates a Cary

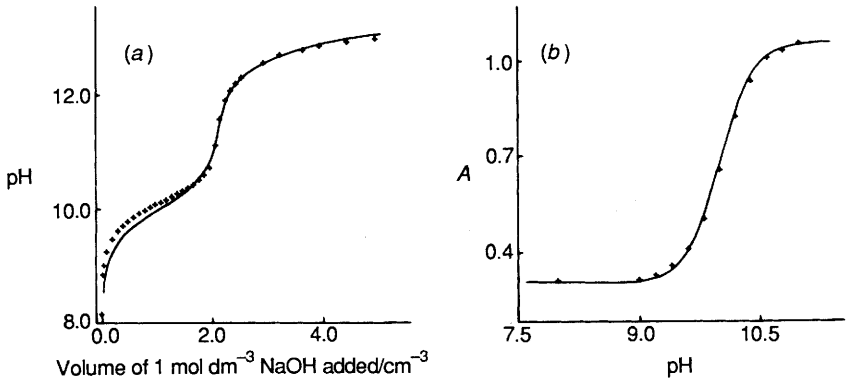


Fig. 1 Determination of K_{41}: (*a*) potentiometric titration of 10^{-3} mol Tz with 1 mol dm^{-3} NaOH; (*b*) spectrophotometric titration of 10^{-4} mol dm^{-3} Tz at λ = 236 nm

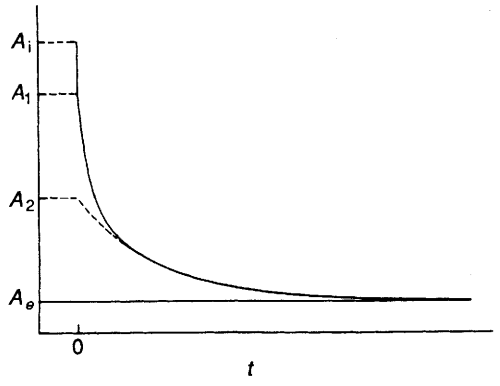


Fig. 2 Change in absorbance A during the progress of the backward reaction, schematically. The reaction is started at t = 0.

Varian 219 spectrophotometer was used. It was equipped with a HP 3478 digital multimeter for the transfer of the data to a computer. The reactions were initiated in two different ways: (*a*) small volumes of a concentrated neutral Tz solution were added to basic buffer solutions in the cuvette (forward reaction); (*b*) buffer solutions and basic solutions and basic Tz solutions were mixed in the cuvette in the ratio 1:1 (backward reaction). The fast reaction rates were measured using a dual beam optical stopped-flow apparatus.[22] Reactions with two relaxation effects were studied using a HP 8452A diode array spectrophotometer, which was equipped with a stopped-flow unit. The stopped-flow measurements were performed by mixing Tz solutions and buffer solutions leading to a final concentration of 10^{-4} mol dm^{-3} Tz. The reaction was performed either by mixing neutral thiazolium solutions with basic buffers or by mixing a Tz solution at pH 12.0 with less basic or acidic buffer solutions. All measurements were performed at 25 °C.

Signal Analysis.—Kinetic measurements were performed under pseudo-first-order conditions and could be fitted to eqn. (2) or eqn. (3) for one or two superimposed relaxation effects, respectively. X_i and τ_i are called relaxation amplitudes and relaxation times, respectively.

$$A_t = X_1 e^{-t/\tau_1} + A_e \quad (2)$$

$$A_t = X_1 e^{-t/\tau_1} + X_2 e^{-t/\tau_2} + A_e \quad (3)$$

Results

Equilibrium Measurements.—The results of the potentiometric and spectrophotometric titrations are shown in Fig. 1. The potentiometric titration reveals the consumption of two equivalents of NaOH per equivalent of Tz. Both titration curves fit quantitatively to the overall reaction (4), with equilibrium constant K_{41} given by eqn. (5).

$$\mathbf{1}^+ \rightleftharpoons \mathbf{4}^- + 2\,H^+ \quad (4)$$

$$K_{41} = \frac{c_4 c_H^2 f^2}{c_1} \quad (5)$$

From least-square fits we obtain $K_{41} = (7 \pm 2) \times 10^{-21}$ mol^2 dm^{-6} for the potentiometric titration and $K_{41} = (10 \pm 1) \times 10^{-21}$ mol^2 dm^{-6} for the spectrophotometric titration. The curves in the figures are calculated with these values. The measurements do not indicate the existence of the uncharged intermediate species **2**, **3** or the ylide **5**.

Forward Reactions.—The kinetics of this reaction were studied by mixing a solution of Tz at pH *ca.* 7 (initial pH) with basic buffer solutions of appropriate composition to obtain a final pH between 9 and 14.5. At the initial pH value Tz exists completely as cation $\mathbf{1}^+$. In all experiments a single relaxation effect is observed, *i.e.* the change of absorbance is described by eqn. (2). The relaxation amplitude X_1 is always equal to the difference between A_i, the absorbance at the initial pH value, and A_e, the absorbance at equilibrium.

Backward Reaction.—This reaction is initiated by mixing a solution of Tz at pH = 12.0 with buffer solutions to obtain a final pH between 0 and 10.8. Now at the initial pH Tz exists almost completely as anion $\mathbf{4}^-$. In these experiments up to three superimposed relaxation effects are observed: (i) a very fast one with a relaxation time below 1 ms, which cannot be resolved by the techniques applied (even at 5 °C), but its existence is clearly deduced from the amplitudes of the slower effects; (ii) a fast step with a relaxation time between 1.8 and 2.7 s; (iii) a slow effect with a relaxation time between 8 and 70 s. The change in absorbance is described by eqn. (3) with $X_1 = A_2 - A_e$ and $X_2 = A_1 - A_2$. A_1 and A_2 are defined in Fig. 2, which schematically shows the changes in absorbance connected with the backward reaction.

The results are summarized in Fig. 3 (relaxation times), 4 and 5 (absorbances A_e, A_1 and A_2). The slow relaxation time τ_1 shows a very pronounced pH dependence. The same values for the relaxation times are obtained for forward and backward reaction in the pH range, where both reactions can be studied (9.2 < pH < 10.8). The fast relaxation effect can only be observed at pH < 8.0. For the forward and backward reaction the final absorbance A_e agrees with the values obtained from equilibrium measurements whereas A_1 and A_2 are only accessible to kinetic measurements.

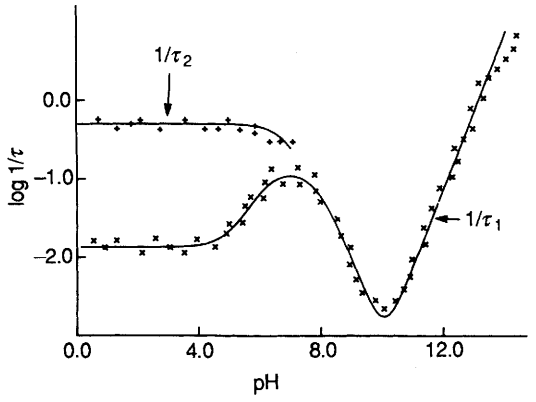


Fig. 3 log $1/\tau$ *versus* pH for the hydrolysis of Tz. Results for forward and backward reactions are included. The curves are calculated with the constants given in Table 1.

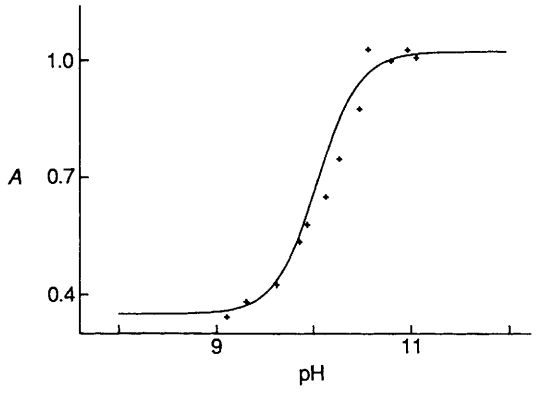


Fig. 4 Final absorbance A_e *vs.* pH (at λ = 236 nm) for the forward reaction of 10^{-4} mol dm^{-3} Tz. The curve is calculated with the constants given in Table 1.

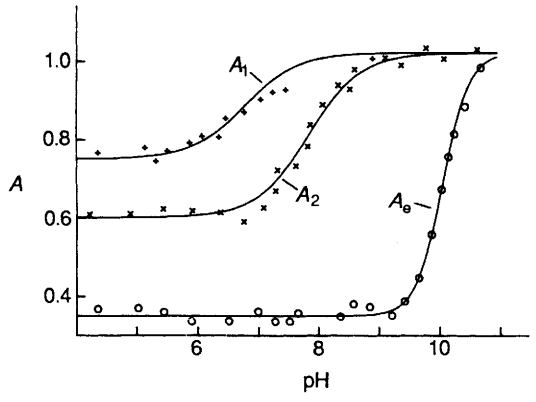


Fig. 5 Absorbances A_1, A_2 and A_e (at λ = 236 nm) as functions of pH measured for the backward reaction. The curves are calculated with the constants given in Table 1.

The progress of the backward reaction was observed with the diode array spectrophotometer, which allows one to measure up to 10 spectra per second. This enabled us to determine the spectrum at $t = 0$ immediately after mixing the solutions before the start of the fast relaxation effect and the spectrum at $t = 3\tau_2$, where the fast relaxation effect is nearly completed, whereas the slow relaxation effect has not yet progressed far. These spectra are shown in Fig. 6 together with those of basic and neutral solutions of Tz.

Evaluation of Relaxation Amplitudes.—The pH dependence of the relaxation amplitudes fits quantitatively to the Breslow scheme (Scheme 1) under the following conditions: (i) step $\mathbf{1}^+ \rightleftharpoons \mathbf{2}$ is rate determining; (ii) step $\mathbf{2} \rightleftharpoons \mathbf{3}$ is faster, but can be

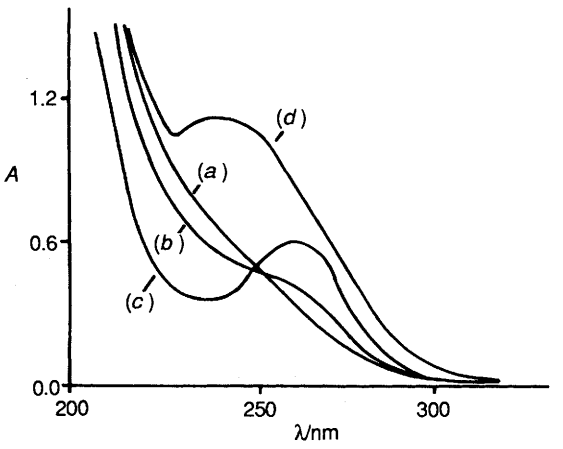


Fig. 6 Spectra obtained when mixing a basic solution of 10^{-4} mol dm^{-3} Tz with 10^{-3} mol dm^{-3} HCl: (*a*) immediately after mixing the solutions; (*b*) at $t = 3\tau_2$; (*c*) at equilibrium; (*d*) spectrum at pH = 12

observed by the stopped-flow technique; (iii) step $\mathbf{3} \rightleftharpoons \mathbf{4}^-$ is too fast to be resolved by the stopped-flow technique; (iv) at all pH values the concentration of the ylide **5** is negligibly small. When studying the forward reaction, we added base to solutions containing the cation $\mathbf{1}^+$. In a slow hydrolysis reaction **2** is formed, which is in rapid equilibrium with **3** and $\mathbf{4}^-$. Therefore only one relaxation effect is observed, and the initial absorbance is due to $\mathbf{1}^+$ only. If the existence of the ylide **5** were to be considered, the absorbance should change in two steps: in the first step the equilibrium between the cation $\mathbf{1}^+$ and the ylide **5** should be established leading to a change in the absorbance, and in the second step the final equilibrium should be reached. Even at pH 14.5 this two-step change has not been observed, and therefore we can assume $pK_{51} > 14.5$ for the dissociation constant of the ylide.

When studying the backward reaction we started with a solution containing the anion $\mathbf{4}^-$. In a very fast reaction the equilibrium between $\mathbf{4}^-$ and **3** is established, connected with a change in absorbance from A_i to A_1. During the fast relaxation $\mathbf{4}^-$ and **3** come to equilibrium with **2**, and the absorbance changes to A_2. During the slow relaxation **2** reacts further to $\mathbf{1}^+$, and the system reaches complete equilibrium. A_1, A_2 and A_e allow us to measure the equilibrium constants for all steps in reaction (6).

$$\mathbf{1}^+ + H_2O \rightleftharpoons \mathbf{2} + H^+ \rightleftharpoons \mathbf{3} + H^+ \rightleftharpoons \mathbf{4}^- + 2H^+ \quad (6)$$

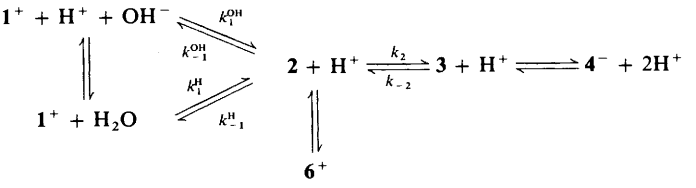
From the pH dependence of A_1 and A_2 we obtain the constants defined in eqns. (7) and (8) respectively. f is the mean activity

$$\frac{c_4 c_H}{c_3} f^2 = K_{43} \quad (7)$$

$$\frac{c_4 c_H}{c_2 + c_3} f^2 = \frac{K_{43}}{1 + K_{32}^{-1}} \quad (8)$$

coefficient of monovalent ions. In Fig. 5 the plots of A_1 and A_2 are kinetic titration curves, and they fit to $K_{43} = (1.7 \pm 0.2) \times 10^{-7}$ mol dm^{-3} and $K_{32} = 0.1 \pm 0.02$. Finally $K_{41} = (8.5 \pm 1.0) \times 10^{-21}$ mol^2 dm^{-6} is determined from A_e, the absorbance at equilibrium, and from eqn. (9) the third equilibrium constant $K_{21} = (5 \pm 2) \times 10^{-13}$ mol dm^{-3} is known.

$$K_{21} = \frac{c_2 c_H}{c_1} = \frac{K_{41}}{K_{43} K_{32}} \quad (9)$$



Scheme 2

Evaluation of Relaxation Times.—The slow relaxation time depends on the pH value in a very characteristic manner, which cannot be explained by reaction (6). For a quantitative evaluation of the relaxation times two further reaction steps have to be introduced into the scheme. (i) The linear increase of the reaction rate with increasing [OH^-] indicates that the thiazolium cation $\mathbf{1}^+$ may also react with a hydroxide ion to form the pseudobase **2**. (ii) The decrease of the slow relaxation rate with the increase of the proton concentration between pH = 7 and pH = 4 indicates the existence of a further species $\mathbf{6}^+$ which is formed by a very fast protonation of **2**. $\mathbf{6}^+$ exists as an intermediate only, *i.e.* K_{61} is much less than unity [eqn. (10)].

$$K_{61} = \frac{c_6}{c_1} \tag{10}$$

This leads to the expanded reaction scheme in Scheme 2. For this system the two relaxation times can be calculated by the following considerations. The fast relaxation effect is observed, when, starting from the anion $\mathbf{4}^-$, the equilibrium is established between **2**, **3**, $\mathbf{4}^-$ and $\mathbf{6}^+$, where step **3** to **2** is rate determining and the cation $\mathbf{1}^+$ does not yet exist. The rate equation for this effect is given as eqn. (11).

$$\frac{\mathrm{d}(c_2 + c_6)}{\mathrm{d}t} = k_{-2}c_3 - k_2c_2 \tag{11}$$

The steps $\mathbf{4}^- \rightleftharpoons \mathbf{3}$ and $\mathbf{2} \rightleftharpoons \mathbf{6}^+$ are fast, and equilibrium between these species can be assumed, *i.e.* eqns. (12) and (13) apply.

$$c_6 = K_{62}c_2c_H \tag{12}$$

$$c_4 = K_{43}c_3c_H^{-1}f^{-2} \tag{13}$$

Since species $\mathbf{1}^+$ has not yet been formed, the mass balance can be written as eqn. (14).

$$c_2 + c_3 + c_4 + c_6 = \text{const.} \tag{14}$$

Finally, we take into account that the solutions are buffered, *i.e.* the reaction proceeds under pseudo-first-order conditions and eqn. (15) holds.

$$c_H = \text{const.} \tag{15}$$

The integration of eqn. (11) under conditions of eqns. (12)–(15) leads to eqn. (16). τ_2 is given by eqn. (17), and c_2' is the

$$c_2 = c_2'(1 - \mathrm{e}^{-t/\tau_2}) \tag{16}$$

$$\frac{1}{\tau_2} = \frac{k_2}{1 + K_{62}c_H} + \frac{k_{-2}c_Hf^2}{c_Hf^2 + K_{43}} \tag{17}$$

concentration of **2** calculated under the condition that the fast relaxation effect is finished and the slow one has not yet started.

During the slow relaxation effect step $\mathbf{1}^+ \rightleftharpoons \mathbf{2}$ is rate determining, and all other steps are at equilibrium. For this effect the rate equation is given by eqn. (18).

$$\frac{\mathrm{d}c_1}{\mathrm{d}t} = k_{-1}^{H}c_2c_H - k_1^{H}c_1 + k_{-1}^{OH}c_2 - k_1^{OH}c_1c_{OH}f^2 \tag{18}$$

Besides eqns. (12) and (13) we have now a third fast pre-equilibrium [eqn. (19)]

$$c_3 = K_{32}c_2 \tag{19}$$

In the mass balance c_1 has to be included, *i.e.* eqn. (20) applies

$$c_1 + c_2 + c_3 + c_4 + c_6 = \text{const.} \tag{20}$$

The integration of eqn. (18) under the conditions of eqns. (12), (13), 15), (19) and (20) yields eqn. (21).

$$\frac{1}{\tau_1} = (k_1^{H} + k_1^{OH}c_{OH}f^2)\cdot\left(1 + \frac{c_H}{K_{21}(1 + K_{32}) + K_{61}c_H + K_{41}c_H^{-1}f^{-2}}\right) \tag{21}$$

The constants involved in this equation can be obtained as follows. (i) The experimental data show, that for pH > 8.5 k_1^H can be neglected in the first parentheses and only the last term counts in the denominator. This leads to eqn. (22) where K_W is

$$\frac{1}{\tau_1} = k_1^{OH}\cdot\left(c_{OH}f^2 + \frac{K_Wf^2c_H}{K_{41}}\right) \tag{22}$$

the ionic product of water. For pH > 8.5 the linear decrease and increase of log τ^{-1} *vs.* pH yield the two constants k_1^{OH} = (7.5 ± 1.0) $dm^3\ mol^{-1}\ s^{-1}$ and $K_{41} = (9 \pm 2) \times 10^{-21}\ mol^2\ dm^{-6}$.

(ii) For 5.5 ≤ pH ≤ 7.5, the '1' in the second parentheses and the last term in the denominator of eqn. (21) may be neglected, and we obtain eqn. (23).

$$\frac{1}{\tau_1} = \frac{k_1^{OH}K_W}{K_{21}(1 + K_{32}) + K_{61}c_H} \tag{23}$$

From the increase of the reaction rate with increasing pH (at pH *ca.* 6) we obtain $K_{61} = (7.0 \pm 1.2) \times 10^{-7}$, and the width of the maximum at pH *ca.* 7 determines $K_{21}(1 + K_{32}) = (6 \pm 3) \times 10^{-13}\ mol\ dm^{-3}$.

(iii) At high proton concentrations τ reaches a limiting value, where eqn. (24) holds with $k_1^H = (1.0 \pm 0.2) \times 10^{-8}\ s^{-1}$.

$$\frac{1}{\tau_1} = \frac{k_1^{H}}{K_{61}} \tag{24}$$

(iv) Eqn. (17) fits to the data of the fast relaxation time, when the first term on the right hand side is neglected. $k_{-2} = (0.5 \pm 0.1)\ s^{-1}$ is obtained at high proton concentrations, where τ_2 is independent of pH. The decrease of the reaction rate at pH *ca.* 7 indicates the value $K_{43} = (2 \pm 1) \times 10^{-7}\ mol\ dm^{-3}$.

It should be mentioned that the three equilibrium constants K_{21}, K_{32} and K_{43} are obtained by evaluating the relaxation amplitudes as well as by evaluating the relaxation times. The agreement between these independently obtained values

Table 1 Rate and acidity constants for the hydrolysis of Tz at 25 °C

K_{21}	5×10^{-13} mol dm^{-3}
K_{31}	5×10^{-14} mol dm^{-3}
K_{43}	1.7×10^{-7} mol dm^{-3}
K_{26}	7×10^{-7} mol dm^{-3}
k_1^{OH}	7.5 dm^3 mol^{-1} s^{-1}
k_{-1}^{OH}	0.15 s^{-1}
k_1^{H}	1.0×10^{-8} s^{-1}
k_{-1}^{H}	2×10^4 dm^3 mol^{-1} s^{-1}
k_2	0.05 s^{-1}
k_{-2}	0.5 s^{-1}

strongly supports the reaction in Scheme 2. Table 1 summarizes all acidity and rate constants obtained from relaxation times and amplitudes and from the titration curves. (All other equilibrium constants can be obtained by combining the acidity constants.) The curves in Fig. 3 are calculated by eqns. (17) and (21) with these constants. The same acidity constants are used for the calculation of the titration curves in Figs. 4 and 5. The curves and the experimental points agree well, except for the relaxation times at very high pH values. Here the deviations are attributed to the difficulty in estimating activity corrections for high ionic strengths.

Finally, the spectra of Fig. 6 should be related to the species in Scheme 2. Spectra (*c*) and (*d*) refer to equilibrium conditions, where only the cation $\mathbf{1}^+$ and the anion $\mathbf{4}^-$ exist, respectively. Spectrum (*a*) is obtained immediately after mixing the basic solution with neutral buffer, and here the Tz exists completely as species **3**. When spectrum (*b*) is measured, equilibrium is reached between **2**, **3** and $\mathbf{6}^+$, and due to the low pH value we observe the spectrum of cation $\mathbf{6}^+$.

Discussion

In this work most information has been obtained from the relaxation amplitudes of pH-jumps produced in stopped-flow measurements. When acid is mixed with basic solutions of Tz, the new equilibrium is established in three steps, which are resolved on the time axis as can be seen in Fig. 2. At the beginning of each experiment Tz exists only in the ring-opened form $\mathbf{4}^-$. In the very fast step the absorbance changes from A_i to A_1, and the corresponding curve in Fig. 5 shows that in this step one proton is consumed. Even at 5 °C this reaction could not be followed by the stopped-flow technique, and therefore we may estimate that at room temperature the relaxation time for this step is smaller than 10^{-3} s. This agrees with the last step in Scheme 1, where a diffusion-controlled rate is expected for the binding of a proton to the sulfur atom of $\mathbf{4}^-$.[23]

In the next step the absorbance changes to A_2. The difference $A_i - A_2$ fits also to the binding of a single proton to $\mathbf{4}^-$. The relaxation time is *ca.* 2 s, and such slow reactions are not observed when protons bind to well solvated atoms of a molecule. Therefore we attribute this step to the ring-closure. As can be seen from curves A_1 and A_2 in Fig. 5, the value of pK_{42} is higher than the value of pK_{43}, *i.e.* compound **2** is more stable than compound **3**. (**2** and **3** have the same stoichiometry.)

In the slowest step the absorbance at equilibrium, A_e, is reached. The steep increase of A_e with pH indicates that two protons are consumed in the overall reaction: one is used in the formation of **2** and **3** and the second one in this last step. A slow reaction is expected for the formation of $\mathbf{1}^+$, since it is connected with the cleavage of a C–O bond. Fig. 5 shows that $\mathbf{1}^+$ is formed at much higher pH values than **2** and **3**, which means that at equilibrium only species $\mathbf{1}^+$ and $\mathbf{4}^-$ exist, as already shown by the titration curves in Fig. 1. Therefore **2** and **3** can be observed only as intermediates in kinetic experiments.

If the pH jump is performed in the opposite direction (*i.e.* base is added to a neutral solution, where Tz exists as the thiazolium cation $\mathbf{1}^+$) a single relaxation effect is observed, since the first step of the overall reaction is rate-determining. The curves in Figs. 4 and 5 are calculated with the constants given in Table 1 and the molar absorbances $\varepsilon_1 = 3500$, $\varepsilon_2 = \varepsilon_6 = 6000$, $\varepsilon_3 = 7500$, $\varepsilon_4 = 10\,300$ mol cm^{-1} dm^{-3}.

Fig. 3 summarizes all relaxation times for the forward reaction, where base is added to a neutral solution and for the backward reaction where acid is added to a basic solution. At pH values where both reactions can be observed, they have the same τ values, since the reaction proceeds under pseudo-first-order conditions. Eqns. (17) and (21) explain the pH dependence of the relaxation times quantitatively, as can be seen by the agreement of the experimental data with the curves calculated using the constants given in Table 1. The values of k_1^H and k_1^{OH} show that the thiazolium ion $\mathbf{1}^+$ forms the pseudobase **2** up to pH = *ca.* 5 predominantly by the reaction with water with the release of a proton, whereas at higher pH values the reaction proceeds predominantly by the direct binding of a hydroxide ion to the C-2 atom.

The narrow maximum of $1/\tau_1$ at pH_{min} = *ca.* 10 is predicted by eqn. (22), which yields $2 \times pH_{min} = -\log K_{41}$, neglecting activity corrections. In order to explain the decrease of the reaction rate with increasing proton concentration at pH = *ca.* 6, we have to introduce $\mathbf{6}^+$ into Scheme 2. This compound reduces the concentration of the reactive species, **2**, in this pH range. $\mathbf{6}^+$ has to be in rapid equilibrium with **2**, since no further relaxation effect is observed in stopped-flow experiments. Therefore in $\mathbf{6}^+$ the proton cannot bind to a carbon atom but only to the nitrogen atom of the thiazolium ring. The width of the maximum of $1/\tau_1$ at pH = *ca.* 7 is larger than the limiting value, which is seen in the minimum at pH = 10. From the width of the maximum we can estimate $K_{21} \cdot (1 + K_{32})$ from eqn. (21).

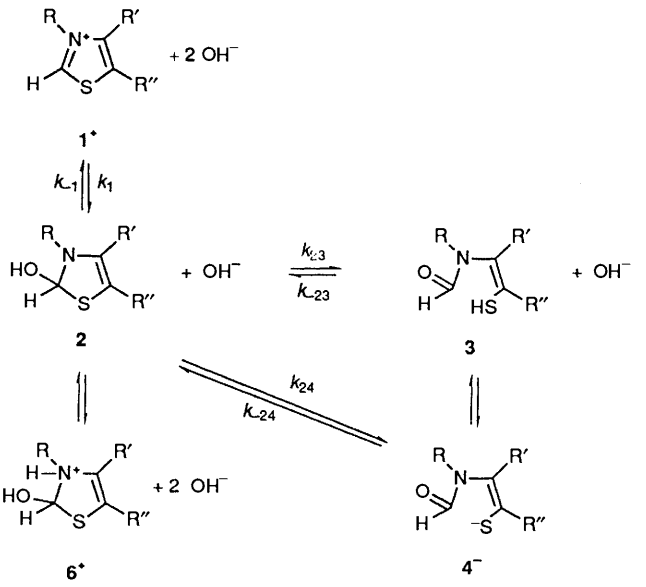
The amplitude of the fast relaxation effect, $A_1 - A_2$ in Fig. 5, becomes very small for pH > 8. Therefore τ_2 can be measured only at lower pH values, where no pronounced pH dependences are observed. In Scheme 1 the ring closure of $\mathbf{4}^-$ occurs *via* the protonation of **3**. However, our measurements cannot differentiate between this path and the direct ring-closure of $\mathbf{4}^-$ connected with protonation of the keto oxygen. Both paths are considered when we replace k_2 and k_{-2} in eqn. (17) by eqns. (25) and (26).

$$k_2 = k_{23} + k_{24} \cdot K_{43} \quad (25)$$

$$k_{-2} = k_{-23} + k_{-24} \cdot K_{43} \quad (26)$$

All our results lead to Scheme 3. We did not observe the formation of the ylide even on addition of 3 mol dm^{-3} NaOH, *i.e.* $pK_a > 15$ for the dissociation of the thiazolium ion (see Scheme 1). This agrees with the values reported by Washabaugh and Jencks,[18] who obtained values in the range 17–19 for different thiazolium ions and for thiamine from NMR exchange measurements.

At this point it has to be emphasized that, from stopped-flow experiments, we may obtain detailed information on the stoichiometry as well as on the rate-determining steps. However, relaxation times and amplitudes yield no direct information on the structure of the species involved. In this respect we have to discuss the spectra in Fig. 6, which have already been assigned to the different species involved. In spectrum (*c*) the absorbance at 260 nm is due to the thiazolium ring of $\mathbf{1}^+$. Spectrum (*b*) of the protonated pseudobase $\mathbf{6}^+$ has to be similar to that of the pseudobase **2**. Otherwise a second step should have been observed at pH = *ca.* 6 in curve A_2 of Fig. 5. Spectra (*a*) for **3** and (*d*) for $\mathbf{4}^-$ differ drastically although the two structures are very similar. One of the referees has pointed



Scheme 3

out that the absorbance of $\mathbf{4}^-$ at 240–260 nm may be due to the enethiolate group, and that this disappears when the group is protonated.

Most authors who have studied the hydrolysis of thiazolium salts have observed only the slow relaxation effect, and they explained their results by the two-step reaction in eqn. (27)

$$\mathbf{1}^+ \underset{k_{-1}^{OH}}{\overset{k_1^{OH}}{\rightleftharpoons}} \mathbf{2} \overset{K_{42}}{\rightleftharpoons} \mathbf{4}^- \qquad (27)$$

where the first step is rate-determining and where the species have the same structures as in Scheme 1. Haake[6] examined the reaction of 3,5-dimethylthiazolium bromide with OH^- using the pH-stat method in the pH range 9–10.5, and the following rate and equilibrium constants were fitted to the experimental data: $k_1^{OH} = 23$ dm^3 mol^{-1} s^{-1}, $k_{-1}^{OH} = 1.5 \times 10^{-2}$ s^{-1} and $K_{42} = 9 \times 10^5$ dm^3 mol^{-1}. Zoltewicz[7] studied the reaction of *N*(1)-methylthiaminium dichloride in the pH range 7–10 and the fit of the experimental data led to $k_1^{OH} = 117$ dm^3 mol^{-1} s^{-1}, $k_{-1}^{OH} = 6.1 \times 10^{-2}$ s^{-1} and $K_{42} = 3.6 \times 10^7$ dm^3 mol^{-1}. Further data for k_1^{OH} (the numbers in brackets are the values of k_1 in dm^3 mol^{-1} s^{-1}) are reported for thiamine[12,13] (20, 13.6), 3-benzyl-4-methylthiazolium bromide[13] (7.8), 3-phenylethyl-4-methylthiazolium bromide[13] (4.7), 3,4-dimethylthiazolium bromide[13,5] (4.0, 15.7), 3,5-dimethylthiazolium bromide[14] (3.0), 3-methylbenzothiazolium iodide[12] (1.6×10^4), 3,4-dimethyl-5-(2-hydroxyethyl)thiazolium iodide[12] (4.0). Vorsanger[8–11] used a slightly different reaction scheme where she considered species **3** instead of **2**.

The following three authors observed two relaxation effects for the backward reaction. Hopmann[15] measured the kinetics of the hydrolysis of thiamine. For the slow reaction $k_1^{OH} = 21$ dm^3 mol^{-1} s^{-1}, $k_{-1}^{OH} = 0.09$ s^{-1} and $K_{42} = 1.8 \times 10^7$ dm^3 mol^{-1} were determined. For the backward reaction he found one fast relaxation effect which he attributed to the reaction step **3** to **2** in eqn. (6) and $k_{-2} = 0.2$ s^{-1} and $K_{43} = 2.4 \times 10^{-7}$ mol dm^{-3} were obtained. El Hage Chahine[17] studied the reaction of thiamine in basic and neutral media. He discussed his results according to eqn. (6), but took into account the second reaction path for step $\mathbf{1}^+$ to **2**. The evaluation of the relaxation times led to $k_1^{OH} = 19.6$ dm^3 mol^{-1} s^{-1}, $k_1^H = 2.15 \times 10^{-4}$ s^{-1} and $k_2 = 8.85 \times 10^{-2}$ s^{-1}. All those evaluations are based upon the Breslow scheme (Scheme 1) and the values of k_1^{OH} obtained for the different compounds are similar (they differ by less than two orders of magnitude). Only Tee[16] explains his results by another reaction scheme. He examined the backward reaction, *i.e.* the pH-jump from a basic thiamine solution to acidic pH values, and he found two relaxation effects. His results are similar to ours, but they cover a smaller pH range. He discusses qualitatively only the relaxation times. He assumes a very fast equilibrium between $\mathbf{4}^-$ and **2** and he assigns the faster relaxation effect to the cleavage of the N–C bond in **2** leading to an amino enethiolester which is present in its protonated form. This is not in agreement with our data which clearly indicate that in the faster relaxation effect an uncharged compound is formed. Moreover it is unlikely that the ring-closure from $\mathbf{4}^-$ to **2** proceeds at a rate which cannot be followed by the stopped-flow technique.

Summarizing all arguments leads to Scheme 3, which for pH > 6 agrees with the Breslow scheme. Step $\mathbf{1}^+$ to **2** is rate-limiting, and step **3** to $\mathbf{4}^-$ is too fast to be followed by the stopped-flow technique. The equilibrium constants K_{21}, K_{32} and K_{43} are determined as well from the relaxation times as from the relaxation amplitudes. The stoichiometry, *i.e.* the state of protonation, of the intermediates is obtained unambigiously from the pH dependence of the relaxation amplitudes, and therefore for pH > 6 further protonation or deprotonation of the intermediates need not be taken into account. Keto–enol tautomerism of the enethiol **3** is conceivable. However, a further relaxation effect should be observed, if this relatively slow reaction[23] were coupled to the fast reaction $\mathbf{4}^-$ to **3**. On the other hand the concentration dependence of relaxation times and amplitudes is not influenced by the existence of isomers, which are in rapid equilibrium with one of the species involved. Therefore such isomers cannot be excluded by our discussion. The cation $\mathbf{6}^+$ had to be introduced, in order to explain the remarkable pH dependence of the relaxation time in acidic solutions at pH < 6, and for pH < 5 the thiazolium ion reacts with water when forming the pseudobase.

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