solution was concentrated in vacuo carefully, and the residue was purified by preparative HPLC (1:2 ether/pentane) to give 3.55 g (70%) of the title compound as a colorless oil: $^1$H NMR (270 MHz) δ 4.90 (m, 1 H), 4.66 (m, 1 H), 2.17 (br s, 1 H), 1.53 (m, 2 H), 0.03 (s, 9 H); IR (neat) 3340, 2955, 1640, 1250, 1164, 1086, 857 cm$^{-1}$; mass spectrum, *m/e* (%) M$^+$ 146 (2.3), 131 (16), 76 (85), 75 (100), 74 (8), 73 (92), 61 (5), 59 (6), 56 (57), 55 (15), 47 (6), 45 (34), 44 (9), 43 (27), 42 (6), 41 (13), 40 (17), 39 (14). Calcd for $C_7H_{14}D_2OSi$: 146.1092. Found: 146.1091.

**Preparation of 1-Acetoxy-1,1-dideuterio-2-((trimethylsilyl)methyl)-2-propene (20) (Table V).** Acetyl chloride (5 mL, 70 mmol) was added dropwise to a solution of the dideuterated alcohol (3.2 g, 22 mmol) in pyridine (9.5 mL, 118 mmol) and 30 mL of dichloromethane at 0 °C. The white cloudy mixture was stirred for 30 min and diluted with ether, washed with saturated sodium bicarbonate, saturated $CuSO_4$, and water, and dried over anhydrous potassium carbonate. The solvent was removed in vacuo to give 3.8 g of residue. It was purified by preparative HPLC (pentane), and the desired product was further purified by Kugelrohr distillation. The title compound (2.4 g, 60%) was obtained as a colorless oil: bp 80 °C (4-5 mmHg); $^1$H NMR (270 MHz) δ 4.89 (m, 1 H), 4.73 (br s, 1 H), 2.09 (s, 3 H), 1.55 (br s, 2 H), 0.04 (s, 9 H); IR (neat) 2955, 1745, 1643, 1370, 1252, 1164, 858 cm$^{-1}$; mass spectrum, *m/e* (%) M$^+$ 188 (9), 173 (6), 146 (18), 145 (34), 131 (30), 129 (6), 118 (12), 117 (63), 113 (8), 91 (7), 83 (5), 77 (13), 76 (32), 75 (90), 74 (53), 73 (98), 72 (16), 71 (6), 70 (8), 69 (9), 62 (6), 61 (26), 60 (17), 59 (33), 58 (22), 57 (37), 56 (84), 55 (46), 54 (20), 53 (10), 47 (23), 46 (15), 45 (97). Calcd for $C_9H_{16}D_2O_2Si$: 188.1197. Found: 188.1198; 91.4% $d_2$, 3.3% $d_1$.

**21**: $^1$H NMR ($C_6F_6$, 100 MHz) δ 7.16 (m, 5 H), 4.64 (s, 0.51 H), 4.56 (s, 0.51 H), 3.96 (m, 1 H), 3.70 (s, 3 H), 2.36 (m, 0.88 H), 1.58 (br s, 2 H), 0.06 (s, 9 H); $^2$H NMR ($C_6F_6$, 15.36 MHz) δ 4.52 (br s, 1.00 D), 2.46 (br s, 1.13 D); mass spectrum, *m/e* (%) (30 eV) M$^+$ 343 (1.2), 156 (98), 145 (12), 126 (11), 125 (100), 110 (11), 97 (13), 78 (17), 77 (86), 75 (18), 73 (58), 72 (22), 69 (23), 59 (52), 57 (17), 51 (24). Calcd for $C_{16}H_{22}D_2O_4SSi$: 342.1284. Found: 342.1276; 97.4 $d_2$, 13.8% $d_1$.

**22**: $^1$H NMR ($C_6F_6$, 100 MHz) δ 7.96-7.60 (m, 5 H), 4.83 (s, 0.05 H), 4.70 (s, 0.50 H), 4.02 (m, 1 H), 3.66 (s, 3 H), 2.60 (m, 0.85 H), 1.76 (s, 3 H); $^2$H NMR ($C_6F_6$, 15.36 MHz) δ 4.56 (br s, 1.00 D), 2.45 (br s, 1.17 D); mass spectrum, *m/e* (%) (30 eV) M$^+$ 156 (19), 129 (100), 125 (21), 97 (26), 77 (22). Calcd for $C_{13}H_{14}D_2O_4S$: 270.0891. Found: 270.0882; 82.1% $d_2$, 9.1% $d_1$.

**23**: $^1$H NMR (270 MHz) δ 7.27-7.02 (m, 4 H), 4.95 (m, 1.30 H), 3.43 (d of t, $J$ = 9.5, 7.3 Hz, 1 H), 3.14 (t of d, $J$ = 7.3, 4.0 Hz, 1 H), 3.03 (d of quintet, $J$ = 17.5, 0.7 Hz, 0.67 H), 2.85-2.65 (m, 1.33 H), 2.39 (br d of d, $J$ = 16.0, 9.5 Hz, 0.65 H); $^2$H NMR ($C_6F_6$, 15.36 MHz) δ 4.01 (br s, 1.00 D), 2.91-2.27 (m, 2.01 D); mass spectrum, *m/e* (%) M$^+$ 202 (100), 201 (9), 174 (16), 173 (10), 159 (6), 146 (8), 131 (7). Calcd for $C_{13}H_{10}D_2O_2$: 202.0960. Found: 202.0963; 89.8% $d_2$, 5.9% $d_1$.

**24**: $^1$H NMR ($C_6F_6$, 100 MHz) δ 7.07 (m, 5 H), 4.80 (m, 1.3 H), 3.20-2.25 (m, 4.5 H), 1.90 (s, 3 H); $^2$H NMR ($C_6F_6$, 15.36 MHz) δ 4.88 (br s, 1.00 D), 2.40-2.70 (m, 2.31 D); mass spectrum, *m/e* (%) (30 eV) 200 (48), 157 (100), 156 (58), 129 (28), 127 (53), 109 (22), 95 (26), 43 (48). Calcd for $C_{14}H_{14}D_2O$: 202.1323. Found: 202.1288.

**25**: $^1$H NMR ($C_6F_6$, 100 MHz) δ 7.40 (m, 5 H), 6.64 (d, $J$ = 16 Hz, 1 H), 4.63 (br s, 1.41 H), 2.76 (m, 2.31 H), 2.28 (m, 1.45 H), 1.78 (br s, 2.57 H); $^2$H NMR ($C_6F_6$, 15.36 MHz) δ 4.62 (br s, 1.00 D), 2.23 (br s, 1.01 D), 1.74 (br s, 1.11 D); mass spectrum, *m/e* (%) (30 eV) 132 (20), 131 (100), 104 (14), 103 (86), 77 (41), 43 (13). Calcd for $C_{14}H_{14}D_2O$: 202.1323. Found: 202.1328; 89.2% $d_2$, 6.2% $d_1$.

**26**: $^1$H NMR ($C_6F_6$, 100 MHz) δ 7.0 (br s, 5 H), 6.5-6.0 (m, 2 H), 4.90 (m, 1.4 H), 4.40 (br s, 1.5 H), 2.24 (d, $J$ = 7 Hz, 1.5 H), 1.92 (s, 3 H), 0.04 (s, 9 H); $^2$H NMR ($C_6F_6$, 15.36 MHz) δ 5.2-4.0 (m, 2.4 D), 2.3 (br s, 1.0 D).

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**Registry No. 1**, 72047-94-0; **6**, 14221-01-3; **7** (X = Y = $Co_2CH_3$), 84681-27-6; **7** (X = $SO_2Ph$; Y = $CO_2CH_3$), 74976-76-4; **7** (X = Y = $SO_2Ph$), 84681-28-7; **8** (X = Y = $SO_2Ph$), 84681-29-8; **87** (X = $COCH_3$; Y = $CO_2CH_3$), 20962-71-4; **8** (X = Y = $-COCH_2C(CH_3)_2CH_2CO-$), 72085-94-0; **8** (X = $SO_2Ph$; Y = $CO_2CH_3$), 74976-77-5; **11**, 74976-79-7; **13**, 84681-30-1; **14**, 84681-31-2; **17**-$PH_3$, 84681-32-3; **20**, 74976-80-0; **21**, 84681-33-4; **22**, 84681-34-5; **23**, 84681-35-6; **24**, 84681-36-7; **25**, 84681-37-8; **26**, 84681-38-9; (*E*)-cinnamaldehyde, 14371-10-9; *n*-heptanol, 111-71-7; ethyl 3-(trimethylsilyl)propionate, 17728-88-0; chlorotrimethylsilane, 75-77-4; ethyl acrylate, 140-88-5; ethyl 2-(hydroxymethyl)-3-(trimethylsilyl)propionate, 84681-39-0; ethyl 2-(trimethylsilyl)propionate, 13950-55-5; paraformaldehyde, 30525-89-4; ethyl 2-(((methylsulfonyl)oxy)methyl)-3-(trimethylsilyl)propionate, 84681-40-3; ethyl 2-methyl-3-(trimethylsilyl)propionate, 74976-84-4; 2-methylene-3-(trimethylsilyl)-1,1-dideuteriopropan-1-ol, 84681-41-4; coumarin, 91-64-5; acetophenone, 98-86-2; benzalacetone, 122-57-6; methyl (phenylsulfonyl)acetate anion, sodium, 60729-65-9; dimethyl malonate anion, sodium, 18424-76-5; bis(phenylsulfonyl)methane anion, sodium, 34782-39-3; methyl 3-oxobutanoate anion, sodium, 34284-28-1; dimedone anion, sodium, 17372-26-8.

# Kinetics and Thermodynamics of the Structural Transformations of Thiamine in Neutral and Basic Aqueous Media. The UV Spectrum of the Tetrahedral Pseudobase Intermediate

**Jean-Michel El Hage Chahine and Jacques-Emile Dubois***

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**Abstract:** pH-jump techniques in chemical relaxation have been used to establish the general mechanism of the structural transformations of thiamine in neutral and mildly basic (pH <11) aqueous media. All the rate and equilibrium constants involved in this mechanism are measured and reported here for the first time. The formation of a tetrahedral pseudobase from thiamine occurs in neutral and acidic media by hydration with a second-order rate constant $k_{H^+}$ = 1.15 × 10$^6$ M$^{-1}$ s$^{-1}$ and an equilibrium constant $K_{H_2O}$ = 1.95 × 10$^{-10}$ M and in basic media by rate-limiting hydroxyl addition with a second-order rate constant $k_{OH}$ = 19.6 M$^{-1}$ s$^{-1}$. The ring opening of the pseudobase is very fast and is always base promoted with a second-order rate constant $k_T$ = 6.75 × 10$^4$ M$^{-1}$ s$^{-1}$ at 5 °C and an equilibrium constant $K_T$ = 1.15 × 10$^{-9}$ M at 25 °C. The pseudobase ring opens probably through basic deprotonation. The pseudobase, which had not been previously detected in the case of thiamine, is isolated kinetically and its UV spectrum is measured point by point. It exists in aqueous solutions between pH 9.2 and 9.4, to the extent of 16% of the overall thiamine concentration.

Ever since Williams' discovery of the structure of thiamine, the antiberiberi vitamin $B_1$, its structural transformations in aqueous media[1-4] and its vitally important action in the decarboxylation of pyruvates[5-8] have been extensively studied. In neutral and

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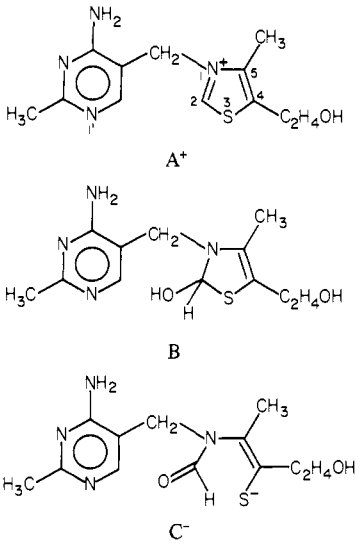
Chart I




mildly basic media (pH <11), thiamine has always been thought to exist in two forms: the cation $A^+$ and the ring-opened thiolate anion $C^-$. The titration of a neutral aqueous solution of thiamine with a standard base is a one-step process requiring 2 equiv of $OH^-$ with an average $pK_a$ of 9.3.[2] Mechanism I, proposed in the literature[2-4] for the transformation from $A^+$ to $C^-$,

Mechanism I

$$A^+ + OH^- \rightleftharpoons B \quad (1)$$

$$B \rightleftharpoons C^- + H^+ \quad (2)$$

implies the existence of a tetrahedral intermediate, pseudobase B (Chart I). Although B was never directly detected in aqueous media, its half-life was estimated by indirect kinetics.[3,4,6] Several authors assumed the rate-limiting step of mechanism I to be the formation of pseudobase B by nucleophilic addition of $OH^-$ to the cation at position 2 while also assuming that the ring opening with proton loss is too fast to be measured by conventional techniques.[4] The rate and equilibrium constants involved in thiamine hydrolysis had not yet been directly measured.

In this work, the use of the pH-jump technique[9,10] in chemical relaxation[11] enables us to propose a mechanism for the structural transformations of thiamine between pH 6.5 and 10.5 and to measure all the rate and equilibrium constants involved in the system. It also enables us to measure indirectly the UV spectrum of the tetrahedral intermediate B which is found in detectable amounts at certain pH values.

## Experimental Section

Thiamine hydrochloride (Aldrich) was crystallized from distilled water, washed with ethanol, dried, and kept under vacuum (lit.[12] mp 248–250 °C).

KCl (Merck suprapur), $NH_4OH$, $H_3PO_4$, and $CH_3CO_2H$ (Prolabo R.P.), and NaOH (Merck titrisol) solutions were used without further purification.

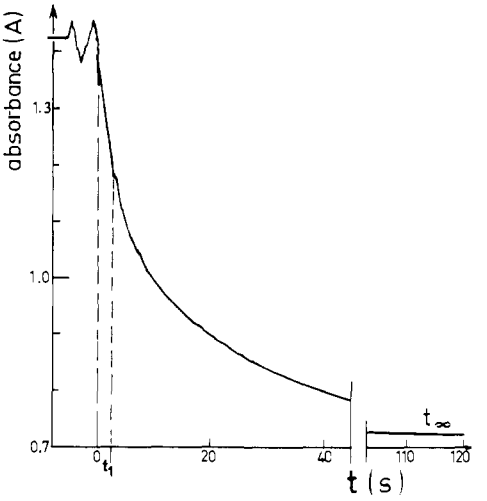



**Figure 1.** Fast pH jump from basic (ca. pH 11.6) to neutral (pH 6.9) at 25 °C. Absorbance change of a thiamine solution at 249 nm (thiamine concentration $c = 1.27 \times 10^{-4}$ M; $t_1$ corresponds to the formation of the kinetic product, and $t_\infty$ to the final equilibrated state with $A^+$ as the thermodynamic product).

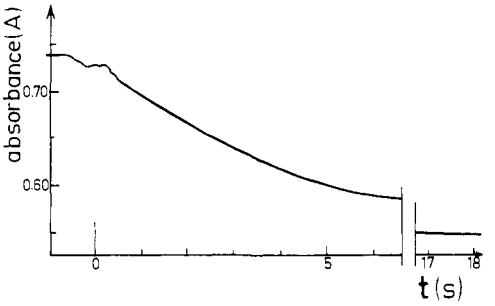



**Figure 2.** Fast pH jump from basic (pH 11.7) to neutral (pH 6.65) at 5 °C. Absorbance change of a thiamine solution ($c = 6.32 \times 10^{-5}$ M) at 249 nm with the formation of the kinetic product only.

**Stock Solutions.** Since basic solutions containing only the thiolate ring-open form are sought, and since thiamine ring opening requires 2 equiv of $OH^-$, basic thiamine solutions ($(2 \times 10^{-5})$–$(1.3 \times 10^{-4})$ M) were prepared with at least 9–10 times the thiamine concentration of NaOH ($(2 \times 10^{-4})$–$(4 \times 10^{-3})$ M). Neutral solutions of thiamine were prepared with the required amount of NaOH to attain pH 7–8. Ionic strength was adjusted to 0.2 M with KCl. These solutions were used only after they had reached total thermal and thermodynamic equilibria (1–2 h). All the solutions were prepared in doubly distilled water.

**Kinetic Measurements.** Relaxation measurements were performed on a Cary 118 or Cary C210 spectrometer, fitted with a thermostated sample cell (2.6-mL capacity, 1-cm optical path), equipped with a magnetic stirring device which allowed for a mixing time of 0.5 s.[9,10] The equilibrium was perturbed by a sudden change in the hydronium ion concentration, while the absorbance was recorded as a function of time. The pH jumps were carried out in the sample cell by injecting microvolumes of (i) concentrated $H_3PO_4$ to mildly basic solutions to achieve stable final pH values, (ii) concentrated $CH_3CO_2H$ to basic thiamine solutions to achieve neutrality, an amount which is less critical with high NaOH concentrations than with low ones (final pH values in this case proved to be quite stable), or (iii) concentrated $NH_4OH$ to neutral thiamine solutions.

**pH Measurements.** The pH was measured before and after the pH jump with a Radiometer pH meter at 5 (±05) °C for fast relaxations and at 25 (±0.5) °C for slow ones. A Metrohm E.A. 125 combined electrode was directly immersed in the sample cell. The buffered solutions used for pH standardization were pH 6.86 and 10.01 NBS standards (Beckman). All values in this paper were adjusted with respect to the activity coefficients of $H^+$ ($\gamma = 0.89$) and $OH^-$ ($\gamma = 0.86$), calculated by the Debye and Hückel relation (25 °C, 0.2 M ionic strength).

**Equilibrium Measurements.** Equilibria were measured at 25 °C by absorption spectroscopy on a Cary 118 spectrometer.

**Signal Analysis.** Reciprocal relaxations were obtained from the experimental signals by the semilogarithmic method with least-squares adjustment.[9,10] The uncertainty varied from 2% for slow signals to 8% for fast ones. *All the observed signals were pure exponentials.*

(1) Williams, R. R.; Ruehle, A. E. *J. Am. Chem. Soc.* **1935**, *57*, 1856.
(2) (a) Maier, G. D.; Metzler, D. E. *J. Am. Chem. Soc.* **1957**, *70*, 4386, 6583. (b) Hopman, R. F.; Brugoni, G. P.; Fol, B. *Ibid.* 1982, *104*, 1341.
(3) Duclos, J. M.; Haake, P. *Biochemistry* **1974**, *13*, 5358.
(4) Zoltewicz, J.; Uray, G. *J. Org. Chem.* **1980**, *45*, 2104.
(5) Yount, R. G.; Metzler, D. E. *J. Biol. Chem.* **1959**, *234*, 738.
(6) Kluger, R.; Chin, J.; Smith, T. *J. Am. Chem. Soc.* **1981**, *103*, 884.
(7) Breslow, R.; McNelis, E. *J. Am. Chem. Soc.* **1959**, *81*, 3080.
(8) Dugas, H.; Penney, G. "Bioorganic Chemistry"; Springer-Verlag: New York, 1981; pp 738–741.
(9) Brouillard, R.; Dubois, J. E. *J. Am. Chem. Soc.* **1977**, *99*, 1359.
(10) Brouillard, R.; El Hage Chahine, J. M. *J. Am. Chem. Soc.* **1980**, *102*, 5375.
(11) (a) Eigen, M.; De Mayer, L. "Techniques of Chemistry"; Weissberger, A., Hammes, G., Eds.; Wiley: New York, 1973; Vol. 6, Part II. (b) Bernasconi, C. F. "Relaxation Kinetics"; Academic Press: New York, 1976.
(12) Steyn-Parvé, E. P.; Monfoort, C. H. "Comprehensive Biochemistry"; Florkin, M., Stotz, E. H., Eds.; Elsevier: Amsterdam, 1963; Vol. 11, pp 1–22.

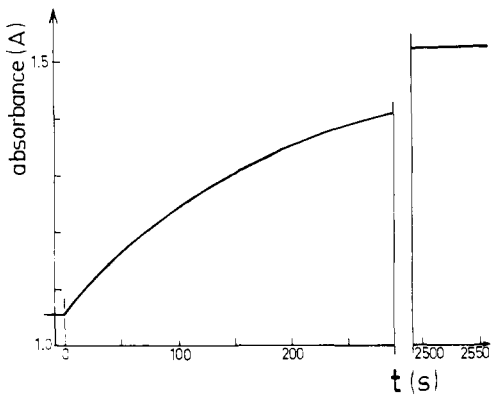



**Figure 3.** Fast pH jump from neutral (ca. pH 7.5) to basic (pH 10.31) at 25 °C. Absorbance change of a thiamine solution ($c = 1.19 \times 10^{-4}$ M) at 233 nm.

## Results

The UV spectra of $C^-$ and $A^+$ are well-known.[2] Two wavelengths were chosen for kinetic runs: 233 nm (the absorption maximum of the $C^-$ form) and 249 nm (the wavelength at which the molecular extinction coefficients of $C^-$ and $A^+$ differ the most).

**Kinetic Phenomena.** When an equilibrated basic solution (pH 11–12, 25 °C, 0.2 M ionic strength) of thiamine (containing only the $C^-$ form) is subjected to a fast increase of its acidity (pH $\simeq$7), two kinetic processes are observed at 249 nm: (a) the first occurs as a sharp decrease of the absorbance with time, (b) the second occurs as a slow exponential decrease of the absorbance. Cation $A^+$ is the final product (Figure 1). A temperature decrease slows the fast process which at 5 °C can be recorded on a normal spectrometer (Figure 2). The amplitudes of both processes seem to be pH independent between pH 6.5 and 7.5.

When an equilibrated neutral solution of thiamine (containing only the $A^+$ form) is subjected to a rapid decrease of its acidity (pH 9.7–10.6), a single kinetic process is observed (Figure 3) at 233 nm. The absorbance increases exponentially with time. The amplitude of this single phenomenon is pH dependent.

*Since all the observed kinetic processes are pure exponentials, chemical relaxation methods can be applied, and the observed signals can be considered as relaxation processes.*[9,10]

**Mechanistic Considerations.** The two relaxation processes observed when a basic thiamine solution is neutralized (Figures 1 and 2) indicate that at time $t_1$ a kinetic product is formed with a relaxation time $\tau_1$. This product gradually evolves into the cation $A^+$, with a relaxation time $\tau_2$ to reach at $t_\infty$ the final equilibrated state. The single relaxation process in basic media (Figure 3) does not involve the accumulation of any kinetic product.

**1. Neutral Media. (a) Fast Relaxation.** The thiolate $C^-$ yields a kinetic product at time $t_1$ (Figures 1 and 2). As $\tau_1$ is too high to be ascribed to a proton transfer,[11,13] the kinetic product cannot be the thiol CH.

$$C^- + H^+ \rightleftharpoons CH \qquad (3)$$

Therefore, at $t_1$ (Figure 1) the thiolate $C^-$ is entirely transformed into the tetrahedral pseudobase B. Two pathways can be considered for the pseudobase ring opening and closure involving a single proton: base-promoted ring opening and acid-promoted closure. However, as a single relaxation time is observed (Figure 2), it can be ascribed to one of the two possible pathways.[14]

$$C^- + H^+ \underset{k_{13}}{\overset{k_{31}}{\rightleftharpoons}} B \qquad K_1 = [C^-][H^+]/[B] \qquad (2)$$

$$B + OH^- \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} C^- \qquad K_{1b} = [B][OH^-]/[C^-] \qquad (4)$$

(13) Eigen, M. *Angew. Chem., Int. Ed. Engl.* **1964**, *3*, 1.

(14) When a single relaxation process is observed, it should be ascribed to a single kinetic phenomenon[11] unless the observed signal (exponential) contains two or more very close relaxations ($\tau' \simeq \tau$, in which case the observed signal would correspond to $\tau$).

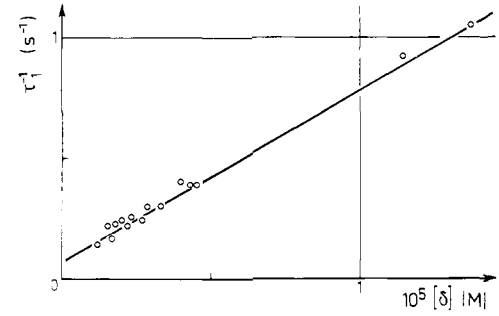



**Figure 4.** Plot of $\tau_1^{-1} = k_{21} + k_{12}\delta$ at 5 °C and 0.2 M ionic strength. Intercept, $(8.85 \pm 0.25) \times 10^{-2}$ s$^{-1}$; slope, $(6.75 \pm 0.15) \times 10^4$ M$^{-1}$ s$^{-1}$; $r = 0.995$; $\delta = [OH^-][B]/([OH^-] + [H^+])$.

With the assumption that $\tau_1^{-1} \gg \tau_2^{-1}$, the substitution method[11] has been used to derive $\tau_1^{-1}$, the reciprocal of the relaxation time of pathways 2 and 4.

In neutral media, before time $t_1$ and after the pH jump, $[A^+] \ll [B], [C]$.

The conservation of matter requires that $\Delta c = 0$ ($c$ is the analytical concentration of thiamine). Thus

$$\Delta[B] + \Delta[C^-] = 0 \qquad (5)$$

Charge conservation implies that

$$\Delta[H^+] = \Delta[OH^-] + \Delta[C^-] \qquad (6)$$

The constant equilibration of water during reactions 2 and 4 implies that

$$[OH^-]\Delta[H^+] + [H^+]\Delta[OH^-] = 0 \qquad (7)$$

So it is easy to show that for unbuffered media[15]

$$\tau_1^{-1} = k_{13} + k_{31}[H^+] + k_{21} + k_{12}\frac{[OH^-]}{[OH^-] + [H^+]}[B] \qquad (8)$$

with $[B] \simeq c$ at $t_1$.[16]

Equation 8 is the general relaxation equation associated with the two possible pathways (reactions 2 and 4). *In order to select the reaction associated with the signal in Figure 2, experimental data were* first *correlated* with eq 8 *by multiple regression* on a PDP-11 computer: part I (the first expression on the right-hand side) of eq 8 was inadequate; $k_{31}$ was negative, $-(5 \pm 4) \times 10^5$ M$^{-1}$ s$^{-1}$, and $k_{12}$ was $(6 \pm 2) \times 10^4$ M$^{-1}$ s$^{-1}$. Then the data were correlated with part II (the second expression on the right-hand side) of eq 8 by linear regression with least-squares adjustments (Figure 4). At 5 °C and 0.2 M ionic strength: $k_{21} = (8.85 \pm 0.25) \times 10^{-2}$ s$^{-1}$, $k_{12} = (6.75 \pm 0.15) \times 10^4$ M$^{-1}$ s$^{-1}$; $K_{1b} = (1.30 \pm 0.20) \times 10^{-6}$ M and $K_1 = K_w/K_{1b} = (1.85 \pm 0.2) \times 10^{-9}$ M, where $K_w = 10^{-14.73}$ M$^2$.

**(b) Slow Relaxation.** Since the fast relaxation (Figures 1 and 2) is ascribed to pseudobase formation from thiolate and since at equilibrium the final product is the cation $A^+$, we suggest that the slow relaxation (Figure 1) be ascribed to the formation of $A^+$ from B. Pseudobase formation occurs via the attack of a hydroxyl ion in basic solutions[17] or via nucleophilic attack of a molecule

(15) Equation 8 is valid for unbuffered media. If the pH jumps performed with acetic acid on basic thiamine solutions do not buffer the media, $H_3PO_4$ neutralization of very low NaOH concentration could have some buffering efect. (It should be noted that when thiamine is dissolved in NaOH solution, a part of the $OH^-$ concentration, equal to twice the concentration of thiamine, would be needed to ring-open the molecule; when an acid neutralizes the basic solution, a part of its concentration is needed to ring-close the molecule.) If thiolate ring closure behaves as if the media were buffered by the pH jumps, eq 8 should be replaced by $\tau_1^{-1} = k_{13} + k_{31}[H^+] + k_{21}[OH^-] + k_{12}$. Neither the general experimental data nor the specific data concerning $H_3PO_4$ jumps performed on mildly basic media fit this equation. So thiamine ring closure behaves as if the media were not buffered.

(16) The amplitudes of the two relaxation processes of Figure 1 are pH independent between pH 6.5 and 7.5. This means that the p$K$ of the ring-opening reaction is higher than 8.5, and that of the pseudobase formation higher than 9.3. Therefore, the concentration of $C^-$ at pH 7 and at time $t_1$ would be lower than 0.01% of the analytical concentration of thiamine.

of water on the cation in acidic media.[10] Near neutrality both cases should be considered. However, since a single relaxation is observed, (Figure 1), it can be ascribed to one of the two possible pathways.[14]

$$A^+ + OH^- \underset{k_{43}}{\overset{k_{34}}{\rightleftharpoons}} B \qquad K_{2b} = [A^+][OH^-]/[B] \qquad (9)$$

$$B + H^+ \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} A^+ \qquad K_2 = [B][H^+]/[A^+] \qquad (10)$$

In the vicinity of neutrality the reciprocal of the relaxation time of pathways 9 and 10 for unbuffered media is expressed as[18]

$$\tau_2^{-1} = k_{23}[H^+] + k_{32} + k_{34}\frac{[OH^-]}{[H^+] + [OH^-]}[A^+] + k_{43} \qquad (11)$$

Since *no experimental data fit eq 11*, pseudobase formation would seemingly be coupled to another equilibrium which could be protonation of cation $A^+$ (Scheme I) with a spectroscopically measured pK of 5.22.

$$A^+ + H^+ \rightleftharpoons AH^{2+} \qquad K = [H^+][A^+]/[AH^{2+}] \qquad (12)$$

Although aqueous solutions when near neutrality contain practically no $AH^{2+}$, equilibrium 12 may have some effect on the rate of pseudobase formation. Reaction 12 is an acid–base reaction; its reciprocal relaxation time is very high compared to $\tau_2^{-1}$. The substitution method can then be used to derive $\tau_2^{-1}$.

Since reaction 12 is in a constant state of equilibrium during reactions 9 and 10:

$$\Delta[AH^{2+}] = \frac{[H^+]}{K}\Delta[A^+] + \frac{[A^+]}{K}\Delta[H^+] \qquad (13)$$

The conservation of matter and charge implies that

$$\Delta[AH^{2+}] + \Delta[A^+] + \Delta[B] = 0 \qquad (14)$$

$$2\Delta[AH^{2+}] + \Delta[A^+] + \Delta[H^+] = \Delta[OH^-] \qquad (15)$$

From eq 13–15 and 7 it is easy to show that

$$\tau_2^{-1} = \left(k_{23}\frac{[H^+]}{4}\left[\frac{([OH^-] + [H^+])(3K + 4[H^+]) + 2[H^+][A^+]}{([OH^-] + [H^+])K + 2[H^+][A^+]}\right] + k_{32}\right) + \left(k_{34}[A^+]\left[\frac{(K + 2[H^+])[OH^-]}{([OH^-] + [H^+])K + 2[H^+][A^+]}\right] + \frac{k_{43}}{4}\left[\frac{([OH^-] + [H^+])(3K + 4[H^+]) + 2[H^+][A^+]}{([OH^-] + [H^+])K + 2[H^+][A^+]}\right]\right) \qquad (16)$$

in which $A^+$ is the final product.[19]

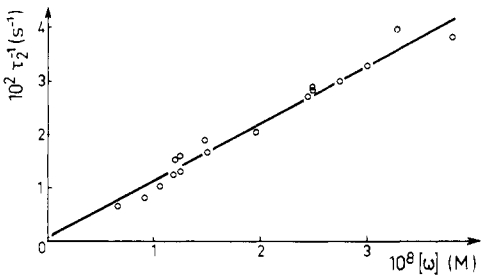



**Figure 5.** Plot of $\tau_2^{-1} = k_{23} + k_{32}\omega$ at 25 °C and 0.2 M ionic strength. Intercept, (2.15 ± 0.15) × $10^{-4}$ $s^{-1}$; slope, (1.15 ± 0.05) $10^6$ $M^{-1}$ $s^{-1}$; $r$ = 0.994; $\omega$ = ([H⁺]/4)[(([OH⁻] + [H⁺])(3$K$ + 4[H⁺]) + 2-[H⁺][A⁺])/(([OH⁻] + [H⁺])$K$ + 2[H⁺][A⁺]).

Relation 16 represents the general relaxation equation associated with the two pathways 9 and 10. After time $t_1$ (Figure 1) a single relaxation mode is observed; to it a single reaction pathway can be associated.[14] *The data, interpreted by multiple linear regression, lead us to retain the reaction associated with the relaxation signals observed.* Experimental data were first correlated with eq 16 by multiple regression. The approximate kinetic parameters showed that part II (nucleophilic addition of $OH^-$ to thiamine, the second expression on the right-hand side) was negligible: the uncertainty on $k_{34}$ and $k_{43}$ was over 100%, and both values were negligible in comparison to $k_{32}$ and $k_{23}$. Linear least-squares regression of the data against part I of eq 16 (acid-promoted dehydration to form thiamine, the first expression on the right-hand side) gave kinetic constants with smaller standard deviations (Figure 5). At 25 °C and 0.2 M ionic strength: $k_{23}$ = (1.15 ± 0.05) × $10^6$ $M^{-1}$ $s^{-1}$, $k_{32}$ = (2.15 ± 0.15) × $10^{-4}$ $s^{-1}$, and $K_2$ = (1.95 ± 0.20) × $10^{-10}$ M. $K_1$ = (1.15 ± 0.15) × $10^{-9}$ M was deduced from the average p$K$ of the overall equilibrium.

**2. Basic Media.** The single slow relaxation observed in basic media (Figure 3) can be ascribed to the addition of $OH^-$ to the cation $A^+$ which, in this case, would be rate limiting. Since reaction 4, which is faster than reaction 9, is always in a state of equilibrium during reaction 9, substitution methods[11] are used to derive the reciprocal relaxation of reaction 9. The medium is considered buffered by aqueous ammonia (p$K_N$ = 9.15 at 25 °C and 0.2 M ionic strength).

Charge conservation implies that

$$\Delta[NH_4^+] + \Delta[A^+] = \Delta[OH^-] + \Delta[C^-] \qquad (17)$$

Matter conservation implies that

$$\Delta[C^-] + \Delta[B] + \Delta[A^+] = 0 \qquad (18)$$

$$\Delta[NH_4^+] + \Delta[NH_4OH] = 0 \qquad (19)$$

The constant equilibration of aqueous ammonia implies that

$$\Delta[NH_4OH] = \frac{[OH^-]}{K_{Nb}}\Delta[NH_4^+] + \frac{[NH_4^+]}{K_{Nb}}\Delta[OH^-] \qquad (20)$$

The constant equilibration of thiamine ring opening implies that

$$\Delta[C^-] = \frac{[B]}{K_{1b}}\Delta[OH^-] + \frac{[OH^-]}{K_{1b}}\Delta[B] \qquad (21)$$

From relations 17, 19, and 20, we can show that

$$\left(1 + \frac{[NH_4^+]}{K_{Nb} + [OH^-]}\right)\Delta[OH^-] = \Delta[A^+] - \Delta[C^-] \qquad (22)$$

in which $K_{Nb}$ = ([$NH_4^+$][$OH^-$])/[$NH_4OH$]. Because the factor [$NH_4^+$]/($K_{Nb}$ + [$OH^-$]) can be neglected under our experimental

(17) Bunting, J. W. "Advances in Heterocyclic Chemistry"; Katritsky, A. R., Boulton, A. J., Eds.; Academic Press: New York, 1979; Vol. 25, pp 1–82.

(18) (a) Equation 11 should be written $\tau_2^{-1} = k_{23}(1 + (K_1/[H^+]))[H^+] + k_{32} + k_{43}(1 + (K_1/H^+)) + k_{34}([OH^-]/([H^+] + [OH^-]))([H^+]^2/([H^+]^2 + K_1(K_1 + H^+)))[A^+]$ However, since $[H^+] \gg K_1$ when the pH is around 7, it simplifies into eq 11. (b) After the pH jump on basic thiamine solutions and after reaction 4 (pseudobase formation), cation $A^+$ is formed from pseudobase B. If the media have been buffered by the pH jumps, eq 11 should be replaced by $\tau_2^{-1} = k_{23} + k_{32}[H^+] + k_{34}[OH^-] + k_{43}$. Neither the general experimental data nor the data concerning $H_3PO_4$ jumps performed on mildly basic media fit this equation. So the cation formation from the pseudobase behaves as if the media were not buffered.

(19) The term in [B] and its factor are neglected in eq 16. As deduced from the value of p$K_1$ at 5 °C and from the average pK at 25 °C, p$K_2$ would be around 9.7–9.8, thereby indicating that B is scarcely present in solutions around pH 7. Moreover, even though [B] cannot always be entirely neglected when compared to [$H^+$], the term with B has been neglected here because, in the equation of $\tau^{-1}$, the factor (($K$ + 2[$H^+$])[$H^+$])/(([$OH^-$] + [$H^+$])$K$ + 2[$H^+$][$A^+$]) which multiplies [B] is very small in comparison to the factor which multiplies [$H^+$], under our experimental conditions. In Figure 1, which is by far the most unfavorable case ($c$ is 1.27 × $10^{-4}$ M, the highest concentration used for this experiment), if p$K_2$ is set at ca. 9.7–9.8, then the term with [B] would represent less than 7% of the term with [$H^+$].

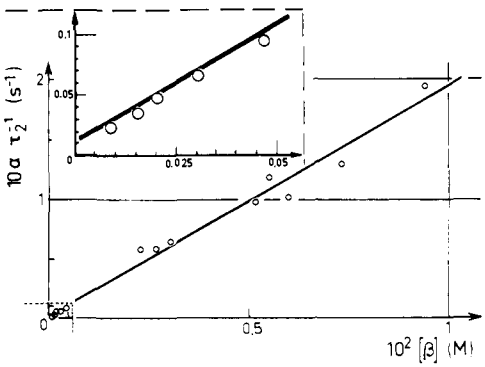



**Figure 6.** Plot of $\alpha\tau_2^{-1} = k_{43} + k_{34}\beta$ at 25 °C and 0.2 M ionic strength. Intercept, (1.15 ± 0.25) × $10^{-3}$ $s^{-1}$; slope, (19.6 ± 1.6) $M^{-1}$ $s^{-1}$; $r$ = 0.993: $\alpha = (K_{1b} + [OH^-] + [B])/(K_{1b} + 2[B])$; $\beta = \alpha[[OH^-] + (K_{1b} + 2[OH^-])/(K_{1b} + [OH^-] + [B])[A^+]]$. The scale is expanded 10 times for the first five points.

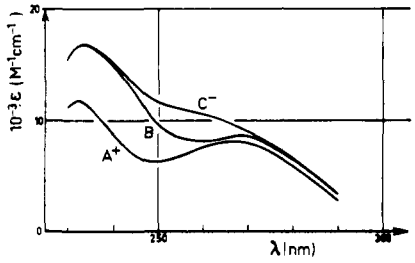



**Figure 7.** Spectra of the three thiamine structures; the molecular extinction coefficient of each species is plotted against the wavelength.

conditions,[20] when the reciprocal relaxation of reaction 9 is derived from relations 22, 18, and 21, it is expressed as

$$\tau_2^{-1} = k_{34}\left([OH^-] + \frac{K_{1b} + 2[OH^-]}{K_{1b} + [OH^-] + [B]}[A^+]\right) + k_{43}\frac{K_{1b} + 2[B]}{K_{1b} + [OH^-] + [B]} \quad (23)$$

in which

$$[B] = \frac{[H^+]K_1}{[H^+]K_1 + K_1K_2 + [H^+]^2}c$$

$$[A^+] = \frac{[H^+]^2}{[H^+]K_1 + K_1K_2 + [H^+]^2}c$$

The experimental data at different thiamine concentrations and different basic pH values fit eq 23 (Figure 6). This fitting, for solutions at 25 °C and 0.2 M ionic strength, gives $k_{43}$ = (1.15 ± 0.25) × $10^{-3}$ $s^{-1}$, $k_{34}$ = (19.6 ± 1.6) $M^{-1}$ $s^{-1}$, $K_{2b}$ = (5.9 ± 0.5) × $10^{-5}$ M and $K_2 = K_w/K_{2b}$ = (1.9 ± 0.2) × $10^{-10}$ M in which $K_w = 10^{-14}$ $M^2$.

**UV Spectrum of Tetrahedral Pseudobase B.** As p$K_1$ is 8.9 when a fast jump from basic to neutral is imposed, at $t_1$ (Figure 1) $C^-$ is completely converted to B. The absorbance at $t_1$ is therefore due entirely to the pseudobase, the kinetic product of equilibrium 4. This experiment, conducted at different wavelengths for identical pH jumps on a basic thiamine solution, allows us to measure the UV spectrum of B. Figure 7 shows the normalized spectra of the three thiamine species ($c$ = 1).

(20) Under the experimental conditions of this work, most of the $\tau_2^{-1}$ measurements in basic media were performed above pH 9.8 (pH 9.8–10.52). Only one $\tau_2^{-1}$ measurement was performed at pH 9.68 ($c = 2 \times 10^{-5}$ M). If we assume that the $NH_4OH$ concentration needed to reach this pH value is ca. 3$c$, the concentration of $NH_4^+$ will be ca. 6.5 × $10^{-5}$ M. Under these conditions, the ratio $[NH_4^+]/(K_{Nb} + [OH^-]) \leq 0.15$. This reasoning is strengthened by the fact that if, at pH 10.5, the $NH_4OH$ concentration needed to reach this pH is ca. 6 × $10^{-4}$ M, this ratio would be below 0.06.

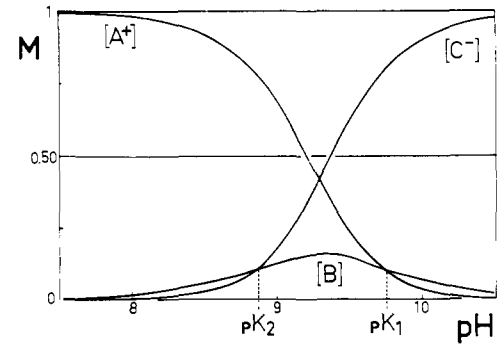
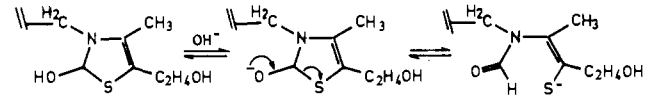
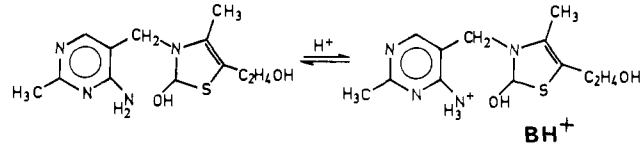



**Figure 8.** Normalized distribution ($c$ = 1) of the different thiamine structures between pH 7.5 and 10.5.

Scheme I



Scheme II




## Discussion

Since mechanism I does not completely explain our results, we have envisaged and supported a new mechanism for the structural transformations of thiamine.

**General Mechanism for the Structural Transformations of Thiamine in Neutral and Mildly Basic Media.**

Mechanism II

$$A^+ + H^+ \rightleftharpoons AH^{2+} \quad (12)$$

$$A^+ + OH^- \rightleftharpoons B \quad (9)$$

$$B + H^+ \rightleftharpoons A^+ + H_2O \quad (10)$$

$$B + OH^- \rightleftharpoons C^- \quad (4)$$

Mechanism II differs from mechanism I in two respects: (i) reaction 12 is involved in reaction 10 which, near neutrality, is due to acid-promoted dehydration of pseudobase B, and (ii) the $OH^-$ promoted ring opening suggests that reaction 4 occurs through the deprotonated pseudobase $B^-$ (Scheme I) as reported for formyl derivatives of tetrahydrofolic acid.[21] Reaction 12 was not investigated further, since it concerns a proton transfer, a kinetic process known to occur in a few microseconds.[13]

As indicated herein, reaction 12 interferes with equilibrium 10, the dehydration of B. This interference might be explained by an intramolecular interaction between the amino group of the pyrimidine ring and OH at the 2-position of the thiazolium ring, and the possible existence of $BH^+$ (Scheme II) as in the case of the internal catalysis involved in the formation and hydrolysis of *N*-isobutylidenemethylamine).[22] Moreover, the thiamine seems to protonate on the N-1′ atom of the pyrimidine.[23] Under these conditions, this protonation can ease the hydration of cation $A^+$.[24] Actually, if the formation of $AH^{2+}$ interferes with the dehydration of pseudobase B, there is no indication that it influences the hydration of $A^+$ because kinetic constant $k_{32}$ is not affected by equilibrium 12.

Reaction 10 is quite slow in neutral media although $k_{23}$ = 1.15 × $10^6$ $M^{-1}$ $s^{-1}$, which is in agreement with the second-order rate

(21) (a) Robinson, D. R. *J. Am. Chem. Soc.* **1970**, *92*, 3138. (b) Robinson, D. R.; Jencks, P. W. *Ibid.* **1967**, *89*, 7089.
(22) Hine, J. *Acc. Chem. Res.* **1978**, *11*, 1.
(23) Cain, A. H.; Sullivan, G. R.; Roberts, J. D. *J. Am. Chem. Soc.* **1977**, *99*, 6423.
(24) As pointing out by one of the referees.

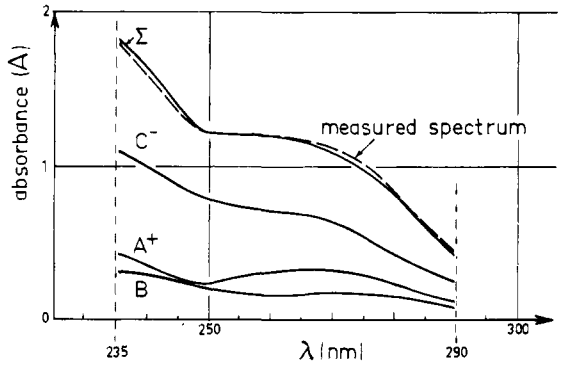



**Figure 9.** Thiamine spectrum reconstructed from the contributions of the calculated spectra of $A^+$, B and $C^-$ (25 °C, pH 9.41, $c = 1.3 \times 10^{-4}$ M; Σ corresponds to the experimental thiamine spectrum measured under the same conditions).

constants ($10^{-3}$–$10^8$ $M^{-1}$ $s^{-1}$) for heterocyclic cation formation from pseudobases.[25,26] Reaction 10 is expected to become very fast in acidic media, whereas reaction 9, pseudobase formation by addition of $OH^-$ on position 2 of thiamine, is shown to be slow and rate limiting in basic media with a second-order rate constant $k_{12} = 19.6$ $M^{-1}$ $s^{-1}$, in agreement with rate values reported for similar pseudobase formation[17] and with the estimated kinetic constants of the rate-limiting step of mechanism I.[6]

The fast relaxation (Figures 1 and 2) can only be due to the ring opening of the pseudobase B because it is too slow to be a proton transfer, even if the transfer is not diffusion controlled.[27] This suggests that equilibrium 3 is relatively acidic, as supposed by Yount and Metzler.[5] If it were not acidic, reaction 3, which is faster than reaction 4, would occur before reaction 4 and its amplitude would be detected by pH jump. The second-order rate constant of reaction 1, $k_{12} = 6.75 \times 10^4$ $M^{-1}$ $s^{-1}$ indicates a fast reaction in basic media. Reaction 4 is not a standard tautomeric ring opening, because it probably involves a proton transfer. The high values of the second-order rate constant is not surprising, because some ring-opening reactions are diffusion controlled.[28]

### Conclusion

*Pseudobase B exists to the extent of 16% of the analytical thiamine concentration between pH 9.2 and 9.5* (Figure 8). This is explained by the fact that the difference between $pK_1 = 8.9$ and $pK_2 = 9.70$ is not high enough to impose a lower concentration of the B form in the basic media *where the observed spectrum of thiamine consists of the contributions of the three different thiamine species, $A^+$, B, and $C^-$* (Figure 9). The pseudobase exists under certain conditions of pH *in detectable amounts*, and it may have some effect on the reactivity of the vitamin, especially via its deprotonated form, $B^-$.

Thiamine is considered as the most important sulfur-containing coenzyme of nonredox enzymatic reactions involving a general acid- or general base-catalyzed proton transfer.[8] In the structural transformations of thiamine itself, a proton transfer is always involved: (i) in the base-promoted ring opening of the pseudobase intermediate, (ii) in the acid–base-promoted hydrolysis and formation of a rather stable pseudobase intermediate from the thiazolium ring. The latter reaction is intimately related to the covalent hydration of electron-deficient heteroaromatic cycles[17] and to Meisenheimer type complex formation. All these reactions involve the formation of a $\sigma$ complex on electron-deficient molecules.[29]

**Acknowledgment.** We are grateful to Dr. J. Lomas and J. Guillerez for helpful discussions.

**Registry No.** $A^+$, 70-16-6; B, 82326-17-8; $C^-$, 23148-74-5.

(25) Bunting, J.; Meathrel, W. G. *Can. J. Chem.* **1974**, *52*, 975.
(26) Shmir, G. L. *J. Am. Chem. Soc.* **1965**, *87*, 2743.
(27) Bernasconi, C. F.; Carré, D. J. *J. Am. Chem. Soc.* **1979**, *101*, 2707.
(28) McClelland, R. A.; Gedge, S. *J. Am. Chem. Soc.* **1980**, *102*, 5838.
(29) Zoltewicz, J. A.; Helmick, L. S.; O'Halloran, J. K. *J. Org. Chem.* **1976**, *41*, 1303.

# Hydroboration Kinetics. 7.[1] Kinetics and Mechanism of the Reduction of Aldehydes and Ketones with 9-Borabicyclo[3.3.1]nonane Dimer

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**Abstract:** The kinetics of the reduction of a number of aldehydes and ketones with 9-borabicyclo[3.3.1]nonane dimer, $(9\text{-BBN})_2$, was studied at 25.0 °C. The reduction of aldehydes and reactive ketones followed first-order kinetic behavior; with less reactive ketones, intermediate or three-halves-order kinetics was observed. Thus the mechanism is very similar to that of the hydroboration of alkenes and alkynes by $(9\text{-BBN})_2$, involving 9-BBN monomer as the intermediate. The relative rates of reduction of representative aldehydes and ketones were determined by the competitive method since the kinetic study could not reveal the effect of the structure upon the reactivity. A comparison with $NaBH_4$ shows that $(9\text{-BBN})_2$ is less susceptible to steric effects, though the same trend is observed with both reagents. Increasing the steric hindrance on one side of the carbonyl function in ketones leads to a modest rate decrease while that on both sides leads to a considerable rate decrease. Electron-withdrawing substituents decrease and electron-releasing ones increase the rate of reduction of aldehydes and ketones. These facts strongly suggest that the boron atom of the reducing species, 9-BBN monomer, is coordinated with the carbonyl oxygen during the reduction process.

The rapid reduction of aldehydes and ketones with diborane at 0 °C was discovered more than 40 years ago.[3] Since then, a wide variety of hydride reducing agents have been developed[4] for the selective reduction of many functional groups. As a result,

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