Destruction of Vitamin B1 by Benzaldehyde. Reactivity of Intermediates in the Fragmentation of *N*1′-Benzyl-2-(1-hydroxybenzyl)thiamin

Ronald Kluger* and Ian F. Moore

Contribution from the Lash Miller Laboratories, Department of Chemistry, University of Toronto, Toronto, Canada M5S 3H6

Received January 18, 2000

Abstract: Thiamin (vitamin B1) combines with benzaldehyde in alkaline solutions to form 2-(1-hydroxybenzyl)-thiamin (HBzT), a reactive intermediate in the thiamin-catalyzed benzoin condensation. In neutral solutions, HBzT fragments into pyrimidine and thiazole constituents by cleavage of the bridging methylene−thiazole bond. The fragmentation is promoted by protonation of the pyrimidine moiety of HBzT. The N1′-benzyl derivative of HBzT (**4** in Scheme 4, BHT) also undergoes fragmentation in neutral and alkaline solutions, consistent with fragmentation being driven by positive charge on the pyrimidine derived from thiamin. Anionic Brønsted bases catalyze the reaction ($\beta = 0.5$, for a series of substituted acetates). The dependence of the observed first-order rate coefficient for fragmentation of BHT on buffer concentration is nonlinear, becoming buffer-independent at concentrations above 0.05 M. This is consistent with a change in rate-determining step with buffer concentration from proton removal to subsequent fragmentation of the conjugate base of BHT. The solvent isotope effect is inverse, also consistent with reversible formation of the conjugate base. Analysis of the kinetic data reveals that the fragmentation step is very fast ($k_f = 1.2 \times 10^5$ s^{-1} at 40 °C). Such a low barrier is consistent with electron-shift mechanisms for the fragmentation step.

Thiamin (vitamin B1) can be used as an alternative to cyanide to catalyze the formation of benzoin from benzaldehyde.[1−4] Breslow's mechanistic analysis of thiamin reactions[5] revealed that catalysis involves base-catalyzed addition of the C-2 conjugate base of thiamin (an ylide) to benzaldehyde, producing the intermediate 2-(1-hydroxybenzyl)thiamin, HBzT (**1** in Scheme 1).

Loss of the C2α proton (derived from benzaldehyde) from HBzT produces an enamine, which is functionally equivalent to a benzoyl carbanion. This adds to the carbonyl group of a second molecule of benzaldehyde, leading to formation of benzoin by elimination of thiamin.

Mieyal and Sable established the existence of the enamine derived from HBzT by observing that the C2α proton of HBzT is exchanged for a deuteron in basic deuterium oxide.[6,7] Similar intermediates also occur in enzymic reactions that involve thiamin diphosphate as a cofactor. For example, the C2α enamine of the diphosphate of HBzT is formed by benzoylformate decarboxylase from the conjugate of benzoylformate and thiamin diphosphate by loss of carbon dioxide (Scheme 2).[8,9] Jordan has been able to generate and observe enamines derived from thiamin adducts.[10−14]

Recent work has shown that there are complications in the reaction possibilities of HBzT. In neutral and acidic solutions, HBzT does not revert to thiamin and benzaldehyde but fragments irreversibly into thiazole and pyrimidine derivatives (Scheme 3).[15,16]

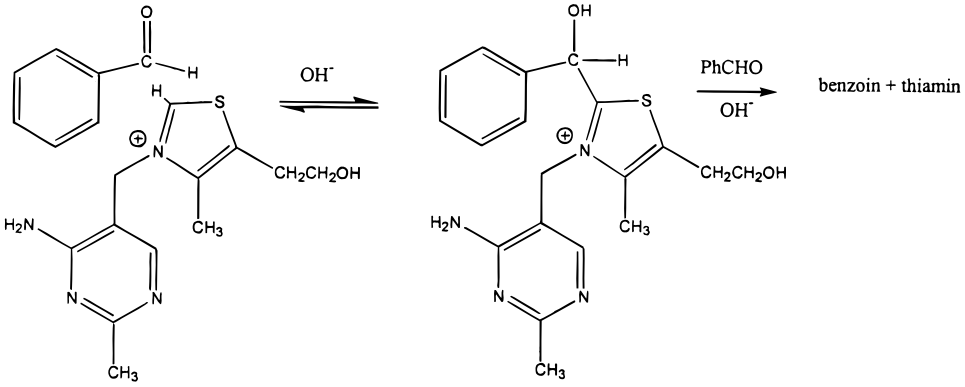
We have been studying the mechanism of the fragmentation reaction and the factors that divert the reaction from the benzoin condensation pathway.[17] A key observation is that the rate of fragmentation of HBzT is proportional to the amount of material that is present as the conjugate acid (protonated at N1′ of the pyrimidine of HBzT). The effect of the positive charge on the pyrimidine ring is consistent with development of carbanionic character at the cleavage site. The observation that the N1′-methyl(pyrimidinium) derivative of HBzT undergoes base-catalyzed fragmentation and no elimination demonstrates the profound effect of the localized charge.[16]

Kinetic patterns of the fragmentation reaction implicate initial removal of the proton at C2α to form the enamine intermediate, as in the benzoin condensation. Exchange of the C2α proton in

(1) Gould, E. S. *Structure and Mechanism in Organic Chemistry*; Holt: New York, 1959; pp 394−397.
(2) Ugai, T.; Tanaka, S.; Dokawa, S. *J. Pharm. Soc. Jpn.* **1943**, *63*, 269−272.
(3) Mohrig, J. R.; Hammond, C. N.; Morrill, T. C.; Neckers, D. C. *Experimental Organic Chemistry*; Freeman: New York, 1998; pp 419−427.
(4) Mohrig, J. R.; Neckers, D. C. *Laboratory Experiments in Organic Chemistry*, 2nd ed.; Van Nostrand: New York, 1973; p 868.
(5) Breslow, R. *J. Am. Chem. Soc.* **1958**, *80*, 3719−3726.
(6) Mieyal, J. J.; Bantle, G.; Votaw, R. G.; Rosner, I. A.; Sable, H. Z. *J. Biol. Chem.* **1971**, *246*, 5213.
(7) Mieyal, J. J.; Votaw, R. W.; Krampitz, L. O.; Sable, H. Z. *Biochim. Biophys. Acta* **1967**, *141*, 205−208.
(8) Dirmaier, L. J.; Garcia, G. A.; Kozarich, J. W.; Kenyon, G. L. *J. Am. Chem. Soc.* **1986**, *108*, 3149.
(9) Hasson, M. S.; Muscate, A.; McLeish, M. J.; Polovnikova, L. S.; Gerlt, J. A.; Kenyon, G. L.; Petsko, G. A.; Ringe, D. *Biochemistry* **1998**, *37*, 9918−9930.
(10) Kuo, D. J.; Jordan, F. *J. Biol. Chem.* **1983**, *258*, 13415−13417.
(11) Jordan, F.; Kudzin, Z. H.; Rios, C. B. *J. Am. Chem. Soc.* **1987**, *109*, 4415−4416.
(12) Barletta, G.; Huskey, W. P.; Jordan, F. *J. Am. Chem. Soc.* **1992**, *114*, 7607−7608.
(13) Jordan, F.; Li, H.; Brown, A. *Biochemistry* **1999**, *38*, 6369−6373.
(14) Jordan, F.; Adams, J.; Farzami, B.; Kudzin, Z. H. *J Enzym. Inhib.* **1986**, *1*, 139−149.
(15) Kluger, R.; Lam, J. F.; Kim, C.-S. *Bioorg. Chem.* **1993**, *21*, 275−283.
(16) Kluger, R.; Lam, J. F.; Pezacki, J. P.; Yang, C.-M. *J. Am. Chem. Soc.* **1995**, *117*, 11383−11389.
(17) Kluger, R. *Pure Appl. Chem.* **1997**, *69*, 1957−1967.

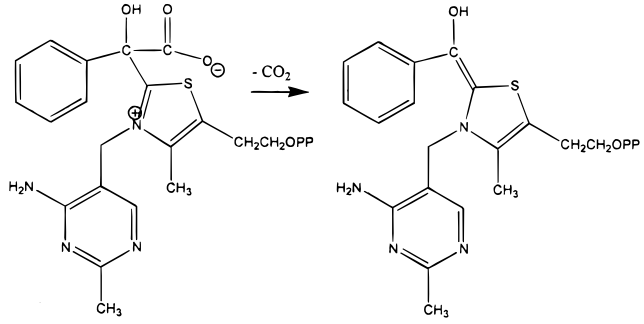
10.1021/ja000194i CCC: $19.00
Published on Web 06/13/2000

Scheme 1



1

Scheme 2



deuterium oxide is faster than the fragmentation process, yet anionic Brønsted bases catalyze the fragmentation.[16] This is kinetically equivalent to fragmentation from the enamine intermediate being catalyzed by Brønsted acids. However, the role for an acid catalyst is not apparent. Thus, we have undertaken further investigations into the reactivity of intermediates in the fragmentation and the roles of catalysts. For these studies we synthesized the N1′-benzyl derivative of HBzT,[18] BHT (**4**, Scheme 4). We have examined the kinetics and reaction patterns of the C2α conjugate base (enamine) of BHT (**5**) over a range of conditions. The results show that the identity of the rate-determining step changes with buffer concentration and that there is a very low barrier to fragmentation from the enamine of BHT.

Experimental Section

Methods. (a) Kinetics of Reactions in Water. The rate of fragmentation of BHT to 2-benzoyl-4-methyl-5-(2-hydroxyethyl)-1,3-thiazole (**2** in Scheme 4) and 4-amino-1-benzyl-2,5-dimethyl-1,3-pyrimidine (**6** in Scheme 4) was monitored with use of buffer solutions maintained at 40.0 °C in a jacketed beaker. The appearance of **2** was followed at 328 nm ($\epsilon = 10\,000$). The pH electrode was standardized against reference buffers at 40 °C. The ionic strength of all reaction solutions was maintained at 0.10 or 1.0 by the addition of sodium chloride or potassium chloride. Data were collected with an interfaced computer. First-order rate constants were calculated from nonlinear regression fitting of the data to the integrated first-order rate expression. Once the kinetic order was determined for a set of conditions, the data for additional sets of reactions were acquired over a relatively short interval and analyzed by the method of initial rates.

(b) Kinetics in Deuterium Oxide. The pK_a′ of potassium dideuterium orthophosphate in deuterium oxide (40 °C, $\mu = 1.0$) was obtained from potentiometric measurements with a glass electrode by using a pH meter, at several buffer ratios (total buffer concentration from 0.10 to 0.15 M). The pD readings were obtained by adding 0.4 to the observed value on the meter.[19] The values of acidity constants for buffers were obtained from published tables.[20]

The rate of exchange of the proton for a deuteron at C2α of BHT was measured by the decrease of the integrated singlet in the ^{1}H NMR at δ 6.6. ^{1}H NMR was also used to follow the fragmentation of BHT as the decrease in the integrated peak at δ 5.9 (due to C6′ H of the pyrimidinium moiety, which is shifted in **6**). The chemical shifts of the C2α and C6′ protons are solvent dependent and assignments were confirmed by HSQC NMR. Integrated areas for the signals of the C2α and C6′ protons were measured in triplicate and compared to the area of the same peaks in the initial spectrum. Data were collected over at least 5 half-lives. First-order rate constants were determined from the slopes of linear regression fits of the data to the integrated rate law. The temperature of the instrument's probe was set at 40.0 °C with calibration using ethylene glycol prior to each run.

Synthesis of *N*1′-Benzyl-2-(1-hydroxybenzyl)thiamin (4). 2-(1-Hydroxybenzyl)thiamin chloride hydrochloride (**1**) was prepared by condensation of benzaldehyde and thiamin.[7,21−23] The product was suspended in ethanol and 1 equiv of sodium hydroxide was added (0.50 M). The solvent was removed under vacuum and the resulting white solid was lyophilized. The dry solid (2.6 g) was dissolved in 6 mL of anhydrous DMSO. The reaction flask was purged with dry nitrogen and benzyl bromide (0.90 mL, 1.2 equiv) was added. The reaction was kept in the dark at room temperature. After 4 days, the reaction mixture was washed with ether and the DMSO layer was separated. The product was precipitated from DMSO by the addition of acetone (15 mL) followed by ether (40 mL). Excess solvent was decanted and residual solvent was removed under high vacuum. The crude product was recrystallized twice from distilled water and converted to the bis-perchlorate salt by the addition of aqueous sodium perchlorate (0.75 g/mL). BHT (0.96 g, 23%) was recovered as a pale yellow solid with mp 172−176 °C dec. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.5 (s, 1H), 8.7 (s, 1H), 7.7 (s, 1H), 7.4−7.1 (m, 10H), 6.9 (s, 1H), 6.3 (s, 1H), 5.37 (dd, 2H, $J = 18$ Hz), 5.22 (dd, 2H, $J = 16$ Hz), 3.7 (m, 2H), 3.0 (m, 2H), 2.4 (s, 3H), 2.3 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 177.7, 161.4, 160.0, 143.1, 141.7, 137.9, 134.4, 133.6, 129.3, 129.2, 129.0, 128.7, 127.8, 127.3, 109.1, 70.0, 59.5, 56.5, 46.5, 29.6, 21.4, 11.3. ^{13}C DEPT NMR (125 MHz, DMSO-d_6): δ 141.7 (+), 129.3 (+), 129.2 (+), 129.0 (+), 128.7 (+), 127.8 (+), 127.3 (+), 70.0 (+), 59.5 (−), 56.5 (−), 46.5 (−), 29.6 (−), 21.4 (+), 11.3 (+). FABMS (high resolution) $[C_{26}H_{30}N_4O_2S]^{2+}$: calcd 462.2092; found 462.2057

Preparative Scale Fragmentation of BHT. A larger scale reaction of BHT was conducted under conditions similar to those of the kinetic

(18) Karimian, K.; Mohanazadeh, F. *Synthesis* **1986**, 1065−1067.

(19) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188−190.

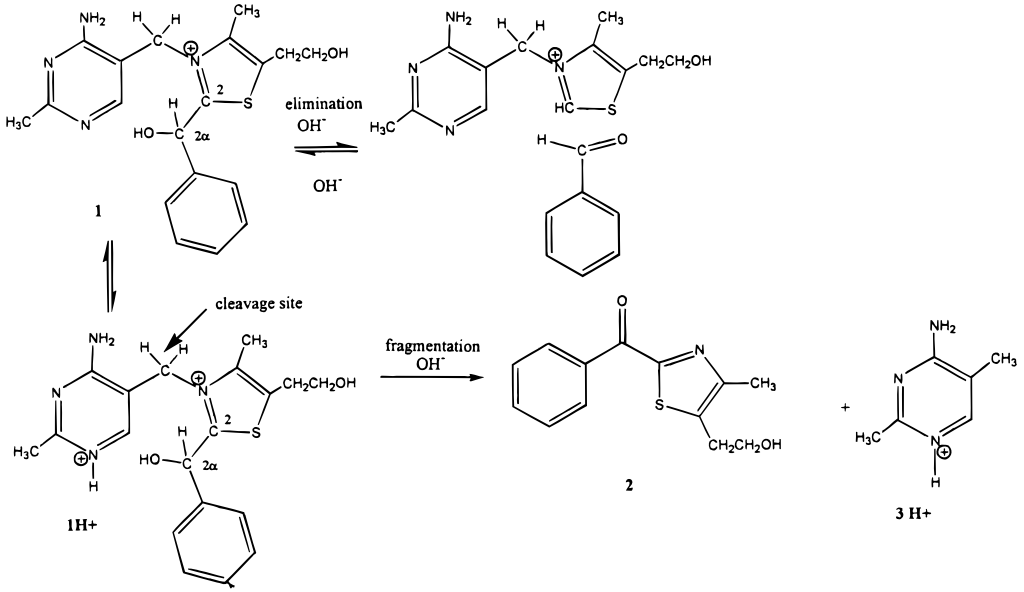
(20) Sober, H. A. *CRC Handbook of biochemistry; selected data for molecular biology*; CRC Inc.: Cleveland, 1968.

(21) Crane, E. J. I.; Washabaugh, M. W. *Bioorg. Chem.* **1991**, *19*, 351−368.

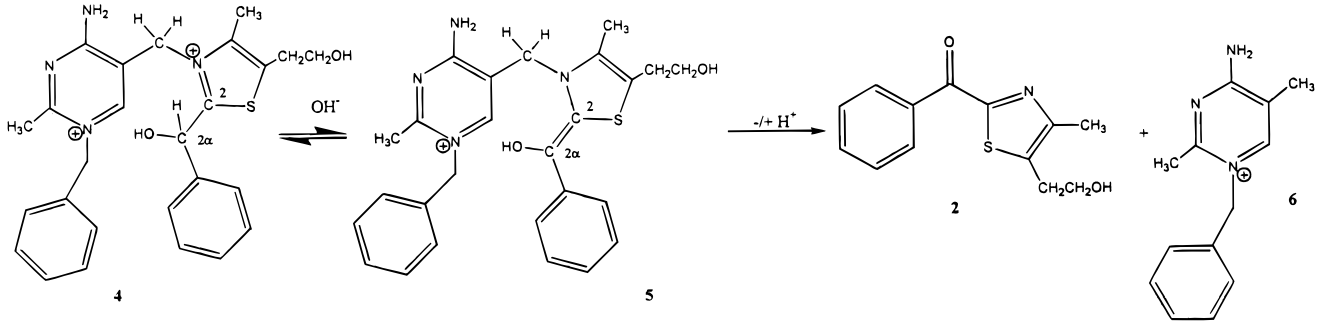
(22) Oka, Y.; Kishimoto, S.; Hirano, H. *Chem. Pharm. Bull.* **1970**, *18*, 527−533.

(23) Oka, Y.; Kishimoto, S.; Hirano, H. *Chem. Pharm. Bull.* **1970**, *18*, 534−541.

Scheme 3



Scheme 4



studies to isolate and identify the products. The bis-perchlorate of BHT (0.095 g) was dissolved in 7 mL of water, final pH 8.0. The solution acidity was maintained by addition of 0.5 M potassium hydroxide from an automated buret. After 1 h the reaction was stopped by addition of hydrochloric acid (1 M) to give an acid concentration in the sample of 0.01 M. The product was extracted with dichloromethane and dried over magnesium sulfate. After the solvent was removed, the yellow oil was purified by flash chromatography on silica gel eluting with a 1:1 combination of hexanes and ethyl acetate (R_f = 0.28) and concentrated (0.03 g, yellow oil). This was identified as the ketone (**2**) formed in the fragmentation of HBzT.[15,16,23] ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.41−8.37 (m, 2H), 7.62−7.45 (m, 3H), 3.87 (t, 2H, J = 6.4 Hz), 3.06 (t, 2H, J = 6.4 Hz), 2.47 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 184.0, 163.7, 152.2, 137.2, 135.5, 133.3, 131.0, 128.3, 62.7, 30.3, 15.3.

The aqueous layer was freeze-dried to yield 0.065 g of a white powder, which was identified as a mixture of *N*1′-benzyl-2,5-dimethyl-4-aminopyrimidine[15,16,22] (**6**) and potassium chloride. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.14 (s, 1H), 8.52 (s, 1H), 8.35 (s, 1H), 7.45−7.27 (m, 5H), 5.39 (s, 2H), 2.51 (s, 3H), 2.08 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.3, 160.5, 145.3, 134.4, 129.1, 128.4, 127.1, 113.5, 56.5, 21.5, 13.4.

Results

The preparation of BHT was accomplished by reaction of HBzT with benzyl bromide in DMSO, following the general procedure of Karimian.[18] Consistent with the reaction patterns seen for *N*1-methyl HBzT,[16] BHT undergoes fragmentation in alkaline solutions (HBzT reverts to thiamin and benzaldehyde under these conditions),[15,21] confirming the importance of the positive charge on the pyrimidine in promoting the fragmentation process. Analysis of the dependence of rate on pH shows that the reaction is first order in hydroxide ion concentration (eight points between pH 5 and 8.5, r = 0.99, Table 1). The fragmentation reaction requires the loss of the C2α proton (derived from the aldehyde group on benzaldehyde). As with *N*1′-methyl HBzT, at the relatively high buffer concentrations necessary for NMR kinetic analysis in deuterium oxide, we observe that the C2α proton exchanges from BHT faster than the overall fragmentation occurs. For reactions followed by UV spectroscopy (with more dilute buffer solutions in water) the basic components of anionic buffers accelerate the fragmentation process (Table 1). Analysis of the dependence of the observed rate on the buffer ratio shows that only the basic component is catalytically active. A Brønsted plot of the kinetic data for dilute formate and substituted acetate buffers (Figure 1) gives a good fit with a slope of Brønsted β = 0.5.

Table 1. Observed First-Order Rate Constants for Fragmentation of BHT in Dilute Buffer

buffer	pH	[buffer], M	k_0 (s^{-1})	k_{B^-} (M^{-1} s^{-1})
acetate	5.00	0.01−0.1	$(3.0 \pm 0.2) \times 10^{-6}$	$(2.3 \pm 0.3) \times 10^{-5}$
succinate	5.90	0.004−0.04	$(2.22 \pm 0.04) \times 10^{-5}$	$(2.7 \pm 0.1) \times 10^{-4}$
phosphate	6.40	0.008−0.08	$(1.2 \pm 0.1) \times 10^{-4}$	$(1.1 \pm 0.2) \times 10^{-3}$
	6.90	0.005−0.04	$(2.4 \pm 0.1) \times 10^{-4}$	$(3.3 \pm 0.2) \times 10^{-3}$
	7.40	0.005−0.04	$(8.4 \pm 0.4) \times 10^{-4}$	$(5.8 \pm 0.8) \times 10^{-3}$
HEPES	7.60	0.01−0.1	$(1.09 \pm 0.07) \times 10^{-3}$	
POPSO	8.10	0.004−0.04	$(3.2 \pm 0.3) \times 10^{-3}$	
bicine	8.60	0.01−0.1	$(1.1 \pm 0.1) \times 10^{-2}$	

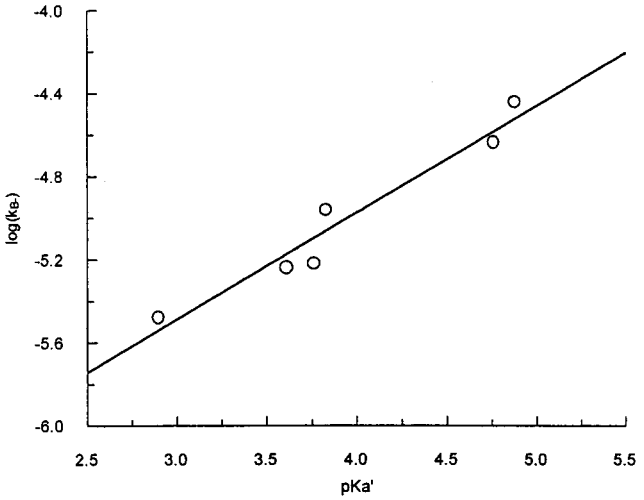


Figure 1. Brønsted plot for fragmentation of BHT catalyzed by substituted acetates and formate at 40 °C and $I = 0.1$.

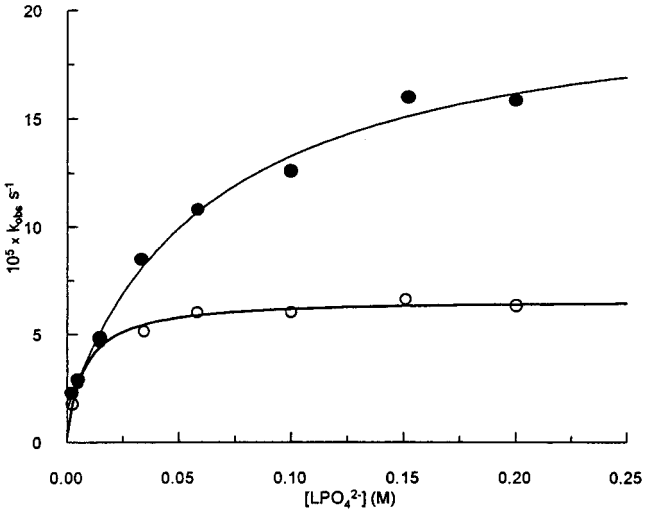


Figure 2. Dependence of the observed first-order rate coefficient for fragmentation of BHT on the concentration of hydrogen phosphate ion in water (○) and deuterium phosphate ion in deuterium oxide (●) at 40 °C, $I = 1.0$. The solid lines are plots of eq 1 ($k_\infty = 6.6 \times 10^{-5}$ $s^{-1}$and $k_o = 2.3 \times 10^{-6}$ s^{-1} in water; $k_\infty = 2.2 \times 10^{-4}$ s^{-1} and $k_o = 1.6 \times 10^{-5}$ s^{-1} in deuterium oxide).

Since UV kinetic data in previous studies were acquired only for dilute buffers and NMR kinetic data only for more concentrated buffers,[15,16] we extended the range of buffer concentrations used for the UV studies to encompass the concentrations used for NMR kinetics. Hydrogen phosphate buffer was used for these studies. The fragmentation is followed in the UV by changes in absorbance at 328 nm due to the formation of the ketone product (**2**). The data obtained from these studies are shown in Figure 2. The observed first-order rate coefficients for fragmentation show a saturating dependence on hydrogen phosphate buffer concentration. The observed first-order rate coefficient in deuterium oxide at the same buffer ratio as in water also shows a linear increase and saturation (Figure 2). The maximum rate in deuterium oxide is significantly larger than that in water, giving an inverse solvent isotope effect ($k_{DOD}/k_{HOH} = 3.0$). The solvent isotope effect in the region where the rate increases linearly with base concentration is also inverse ($k_{DOD}/k_{HOH} = 1.3$ measured at the lowest buffer concentration).

Table 2. Derived Rate Constants and Ratios for Fragmentation of BHT in Water and Deuterium Oxide[a]

solvent	k_B (M^{-1} s^{-1})	k_{BH}/k_f (M^{-1})	k_w/k_f
H_2O	7.4×10^{-3}	56	0.5
D_2O	3.1×10^{-3}	6.9	0.1

[a] Conditions described in the caption to Figure 2

Table 3. Second-Order Rate Constants for General Base Catalysis

buffer	pK_a	k_{B^-} (M^{-1} s^{-1})
chloroacetate	2.90	3.11×10^{-6}
methoxyacetate	3.61	5.75×10^{-6}
formate	3.77	6.07×10^{-6}
glycolate	3.83	1.07×10^{-5}
acetate	4.77	2.30×10^{-5}
propionate	4.88	3.60×10^{-5}

Discussion

The plot in Figure 2 provides a unifying basis for understanding catalytic steps in the fragmentation of BHT and this extends to HBzT. The UV kinetic data for the rates of fragmentation at the high buffer concentrations are clearly invariant while at low concentrations there is a linear dependence. Earlier NMR studies at high buffer concentrations revealed that the rate of fragmentation does not change with changes in buffer concentration.[16] We can see from the present results that the invariant rate was observed in the earlier studies because the high concentration caused the proton removal step to be faster than the subsequent uncatalyzed fragmentation step.

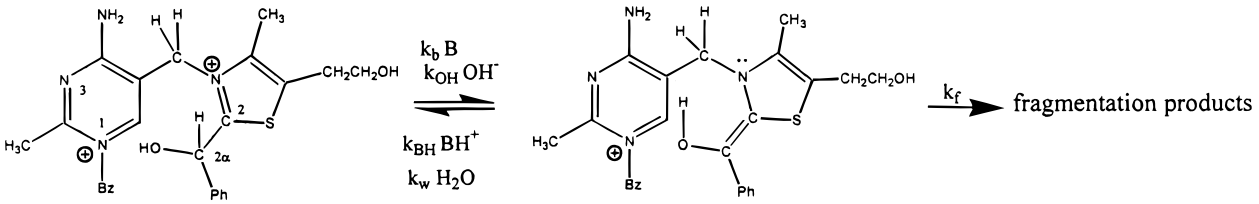
The change in the slope of the plot of observed rate coefficient versus buffer concentration for fragmentation of BHT (Figure 2) is consistent with a single mechanism having a change in rate-determining step with increasing buffer concentration. At low concentrations, the reaction is subject to Brønsted base catalysis but at high buffer concentrations this becomes limited by a subsequent process that does not involve a rate-determining proton transfer. Thus, the rapid proton exchange of BHT (compared to fragmentation) observed by NMR occurs in solutions where the observed rate coefficient for fragmentation is not increased by additional buffer concentrations. (Since the fragmentation process in HBzT[15] is much slower than proton exchange at C2α, it is reasonable that the early discovery of the exchange reaction at C2α was not complicated by fragmentation.[7]) Thus, for BHT, removal of the C2α proton is the step that is susceptible to general base catalysis in the fragmentation process, consistent with the slope of the Brønsted plot of 0.5. The curvature of the buffer plot occurs at the buffer base concentration where the rate of the reverse reaction, protonation of the enamine by the acid form of the buffer, is equal to the rate of fragmentation of the intermediate that is unlikely to be catalyzed. (Since the enamine is formed as a reactive steady-state intermediate we do not expect to be able to observe it as has been done for nonaqueous conditions where the opportunity for proton transfer is suppressed.[11,12,24])

The Brønsted plot (Figure 1) for general base catalysis of fragmentation of BHT by the buffers listed in Table 2 has a slope of 0.5, consistent with a rate-determining step in which a proton is abstracted from a carbon acid having a delocalized conjugate base.[25] The rate constant for proton removal by hydroxide from BHT is 1×10^3 M^{-1} s^{-1}. The data point for catalysis by hydroxide ion is well above the extrapolated Brønsted plot for carboxylate buffers. This is consistent with a rate-determining proton transfer from carbon as the solvent lyate

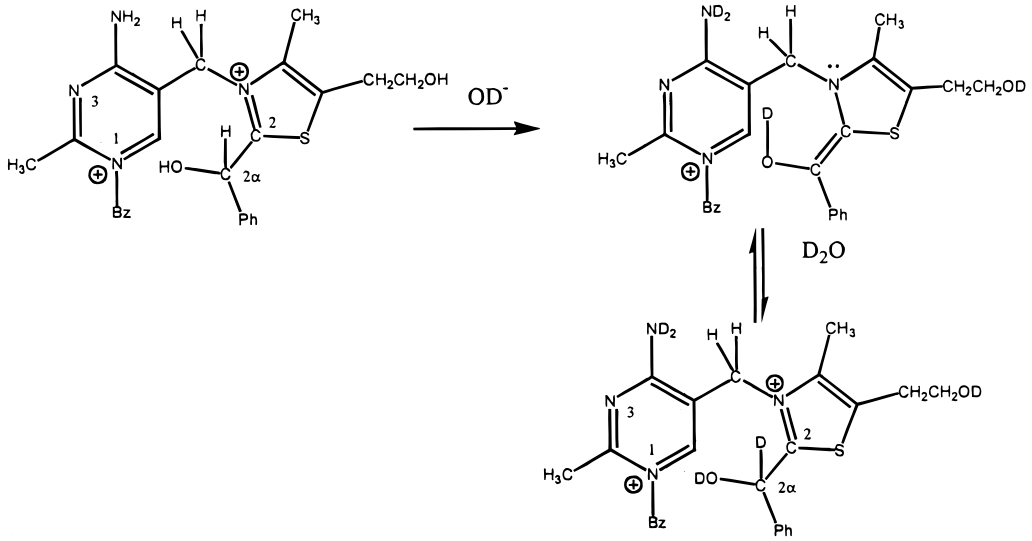
(24) Barletta, G. L.; Zou, Y.; Huskey, W. P.; Jordan, F. *J. Am. Chem. Soc.* **1997**, *119*, 2356−2362.

(25) Bell, R. P. *The Proton in Chemistry*, 2nd ed.; Cornell University Press: Ithaca, New York, 1973; pp 194−225.

Scheme 5



Scheme 6



ion can react through the solvation shell while the buffer components must react directly.[25,26]

These results can be accommodated by a mechanism in which removal of the C2α proton from BHT is rate-determining for fragmentation in dilute buffers but a subsequent step is rate-determining at higher buffer concentrations. The dependence of the observed first-order rate constant on buffer components and solution acidity according to the mechanism in Scheme 3 under steady-state conditions is given by eq 1 (terms shown in Scheme 5).

$$k_{obs} = (k_B[B] + k_{OH}[OH^-])\, k_f/(k_{BH}[BH] + k_w + k_f) \quad (1)$$

Following the method outlined by Keefe and Jencks,[27] nonlinear regression of the data in Figure 2 with eq 1 was used to find the rate constant at zero buffer and at saturation. We obtain $k_B = 7.4 \times 10^{-3}$ M^{-1} s^{-1} for removal of the C2α proton from BHT by the basic component of the buffer as well as the rate constant ratios, $k_{BH}/k_f = 56$ M^{-1} and $k_w/k_f = 0.5$. With use of a specific value for the concentration of water of 55.5 M and $k_w = k_{HOH}$[HOH], then $k_{HOH}/k_f = 9 \times 10^{-3}$ M^{-1} and $k_{BH}/k_{HOH} = 6 \times 10^3$. Table 2 contains a summary of the derived kinetic parameters for reactions in water and deuterium oxide.

From the values of the rate constants in the ratio expression in Table 1 and the reported pK_a of 15.4 for C2α of the related methoxybenzyl methylthiazolium salts[28] (which we assume will be similar for BHT), we obtain $k_{BH} = 6.7 \times 10^6$ M^{-1} s^{-1} and $k_f = 1.2 \times 10^5$ s^{-1} by substitution into eq 1. The ratio establishes that the fragmentation process from the enamine intermediate (**5**) associated with k_f is very fast, competing with the kinetically significant rate for protonation of the conjugate base. The inverse solvent isotope effects on k_B provide further insights. At low buffer concentrations, proton removal is rate determining. This is consistent with a normal isotope effect on dissociation of the conjugate acid of the catalytic base. The high inverse solvent isotope effect at saturation is consistent with deuteration of the conjugate base of BHT being expectedly slower than is protonation of the same species in Scheme 7. (The reverse reaction thus displays a normal solvent isotope effect, similar to what was reported by Keefe and Jencks in their study of $E1_{CB}$ reactions.[27,29])

The low barrier to the unimolecular fragmentation of the ionized intermediate suggests that such a process would be a problem in benzoylformate decarboxylase where the conjugate base of the pyrophosphate of HBzT is formed by the loss of carbon dioxide from the precursor derivative of thiamin diphosphate (Scheme 2). A low net dielectric of the medium at the active site would accelerate decarboxylation and suppress fragmentation because protonation of the pyrimidine would be suppressed (the conjugate acid is a dication).

The mechanism of the fragmentation process of the conjugate base of BHT remains an interesting question. We now know that it is not subject to Brønsted acid−base catalysis, so it is unlikely to involve kinetically significant proton transfers. Since the conversion to the fragmentation products is very fast, the formation of free forms of highly energetic intermediates is unlikely and may involve assisted proton transfers.[27] The electron-shift arrows in Scheme 7 summarize the steps in a six-electron electrocyclic process (related to a [1,5]-sigmatropic rearrangement), which could be consistent with the very low activation barrier for the reaction. There are intriguing alternative

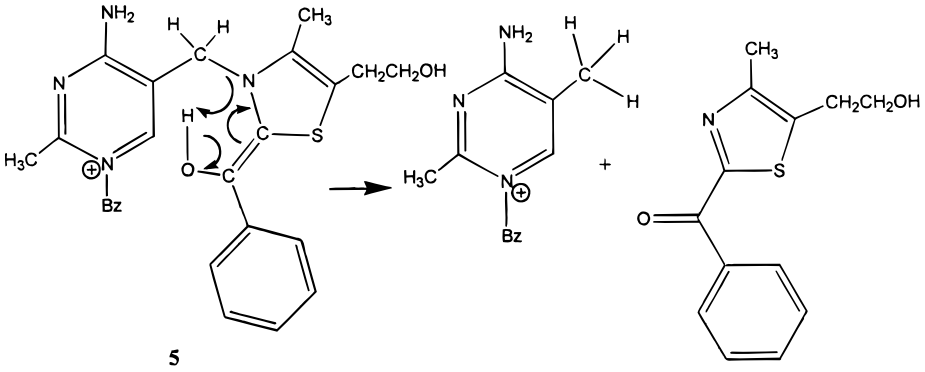
(26) Kluger, R.; Wong, M.; Dodds, A. K. *J. Am. Chem. Soc.* **1984**, *106*, 1113−1117.

(27) Keeffe, J. R.; Jencks, W. P. *J. Am. Chem. Soc.* **1983**, *105*, 265−279.

(28) Bordwell, F. G.; Satish, A. V.; Jordan, F.; Rios, C. B.; Chung, A. C. *J. Am. Chem. Soc.* **1990**, *112*, 792−797.

(29) Keeffe, J. R.; Jencks, W. P. *J. Am. Chem. Soc.* **1981**, *103*, 2457−2459.

Scheme 7



processes that can accomplish the transformation, including a suggestion by a referee that involves addition of the carbanion at C2α to the adjacent pyrimidinium ion, followed by cleavage in analogy to the mechanism by which sulfite cleaves thiamin through initial addition to the pyrimidine.[30]

Conclusions

BHT, like the *N*1′-methyl derivative of HBzT,[16] does not release thiamin in neutral and alkaline solutions but fragments into thiazole and pyrimidine components. Our kinetic study of fragmentation of BHT over a wide range of concentrations establishes that the observed buffer catalysis arises from the first step, transferring the C2α proton to a base. Catalysis of a similar step will explain the general base catalysis observed with fragmentation of hydroxybenzylthiamin[15] and its *N*1′-methyl analogue.[16] Therefore, the fragmentation process from the conjugate base probably occurs without catalysis. The inverse solvent isotope effect on the rate at saturating buffer concentration confirms the existence of a carbanion equivalent as an intermediate. The nonlinear dependence of the rate of fragmentation on buffer concentration permits an analysis that shows that the steps leading from the conjugate base to the fragmented products are very fast, suggesting the possibility of an electrocyclic mechanism that splits the thiazole and pyrimidine components. Finally, it is clear that enzymes that involve reactions via similar intermediates need to provide a means to avoid the fragmentation reaction. Structural information suggests that the active site of benzoylformate decarboxylase is relatively nonpolar.[9] This environment will lower the apparent basicity of the pyrimidine derived from hydroxybenzylthiamin diphosphate. Since the fragmentation is critically dependent on the pyrimidine being positively charged,[16] the normal catalytic process may avoid competition from fragmentation.

Acknowledgment. We thank the Natural Sciences and Engineering Research Council of Canada for support through an operating grant and Dr. John Pezacki for helpful discussions. Ian Moore is the recipient of an Ontario Graduate Scholarship in Science and Technology.

JA000194I

(30) Zoltewicz, J. A.; Uray, G. *Bioorg. Chem.* **1994**, *22*, 1−28.