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Decomposition of 2-(1-Hydroxybenzyl)thiamin in Neutral Aqueous Solutions: Benzaldehyde and Thiamin Are Not the Products

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It has been reported that in neutral aqueous solutions, the adduct of benzaldehyde and thiamin, 2-(1-hydroxybenzyl)thiamin, HBzT **(2)**, undergoes general base-catalyzed reversion to thiamin and benzaldehyde (E. J. Crane, III, and M. W. Washabaugh (1991) *Bioorg. Chem.* **19**, 351). An unusual mechanism had been invoked to explain the kinetic observations. In the present study, it is shown that in solutions of pH < 8, HBzT fragments at the bridge methylene group to 2,5-dimethyl-4-amino-pyrimidine **(3)** and 2-benzoyl-5-(2-hydroxyethyl)-4-methylthiazole **(4)**, not thiamin and benzaldehyde (which are the products at higher pH) (Scheme 3). Buffer catalysis is not observed where the products are thiamin and benzaldehyde. In neutral solution under conditions in which the products are **3** and **4**, the reaction is general base-catalyzed. It is likely that catalysis assists the cleavage of the methylene bridge. Mechanistic proposals based on the elimination of benzaldehyde from HBzT in neutral and acidic solutions should be reconsidered. © 1993 Academic Press, Inc.

The adduct of thiamin **(1)** and benzaldehyde, 2-(1-hydroxybenzyl)thiamin (HBzT, **2**), is the initial covalent intermediate in the thiamin-catalyzed formation of benzoin (Scheme 1). The intermediate was proposed by Breslow to explain thiazolium salt catalysis of the benzoin condensation (*1*).

Crane and Washabaugh reported that the conversion of HBzT to thiamin and benzaldehyde is general base-catalyzed, with $\beta = 0.6$ (*2*). Based on the structures of the reactants and products, they proposed a novel mechanism for general acid catalysis of the breakdown of the benzyl alkoxide derived from HBzT in which a proton is transferred into the carbon–carbon (C–C) bond in concert with cleavage of that bond. Steric and electronic limitations led to the suggestion that the proton transfer involves remote orbitals of the thiazolium ring (Scheme 2). The overall cleavage is analogous to the formation of intermediates in almost every thiamin-dependent enzyme, and the general implications of such a mechanism are significant.

Catalysis in which proton transfer is concerted with carbon–carbon bond cleavage is the expected result when a stepwise process is not possible (*3*). Yet, this case involves species which have significant lifetimes and moderate relative energies (*4*). To accommodate this observation within the generally understood framework, Crane and Washabaugh invoke a critical preassociation complex which avoids a

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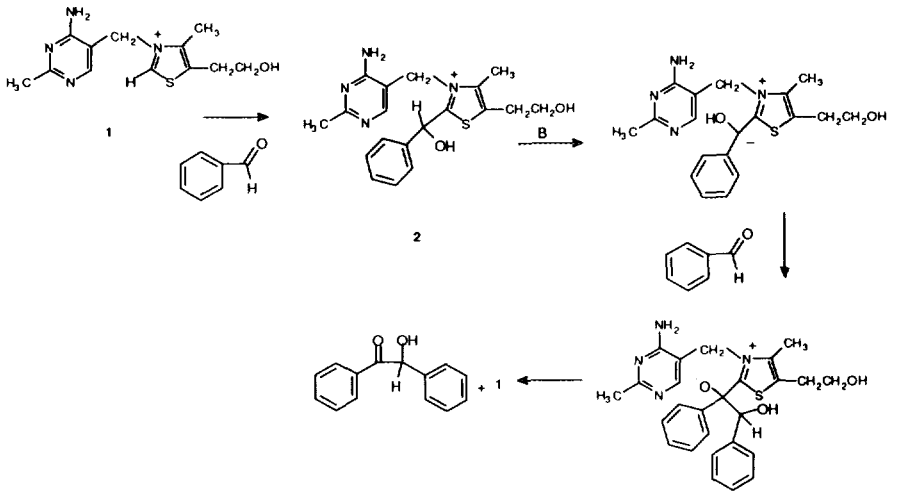


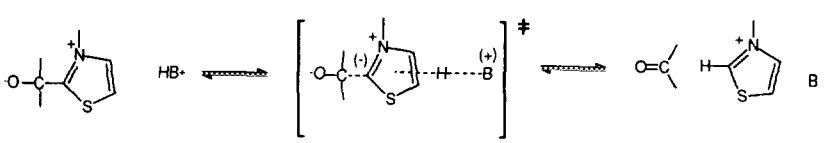
SCHEME 1. Formation of HBzT from thiamin and benzaldehyde; benzoin condensation.

freely diffusing thiamin ylid (the conjugate base formed by transfer of the C-2 proton of the thiazolium ring) as an intermediate (*2*). Generalization of this result might suggest that the thiamin ylid is too reactive to permit it to diffuse once formed. In the case of the decomposition of the pyruvate adduct of thiamin, the observation of only specific base catalysis was taken as an indication that the immediate products can exist and equilibrate with solution (*5*).

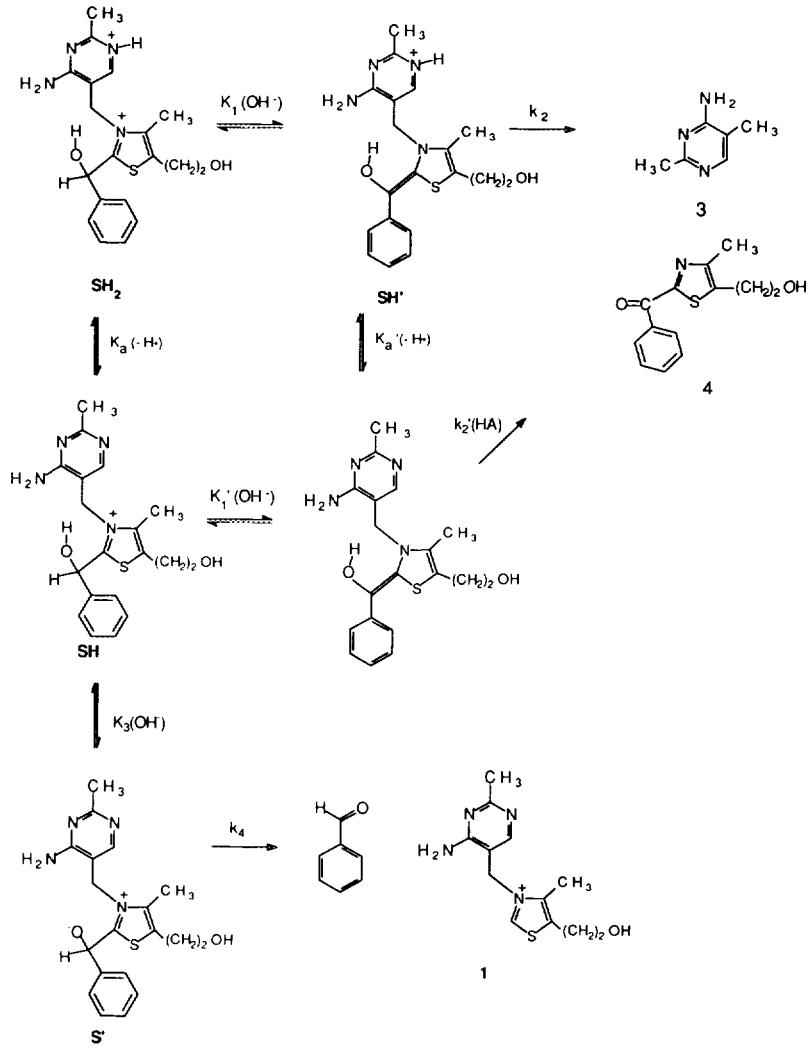
It had been noted by Oka and co-workers in 1970 that HBzT decomposes to benzaldehyde and thiamin in alkaline methanol, but there is a very different set of products from reaction in neutral methanol containing triethylamine (*6*, *7*). We now report that such a contrast applies for reactions in water as well (Scheme 3), accounting for the unusual kinetic patterns seen by Crane and Washabaugh (*2*) which had been attributed to the formation of benzaldehyde and thiamin. In acidic and neutral solutions, the products are **3** and **4**, while in alkaline solution, the products are thiamin and benzaldehyde.

MATERIALS AND METHODS

Ultraviolet kinetics were performed in 1-cm quartz cuvettes kept at constant temperature with a recirculating water bath in the jacketed cell holder of a Perkin–Elmer Lambda 2 or Lambda 19 UV/Visible spectrophotometer. Data were



SCHEME 2. Proposed acid catalysis of C–C bond cleavage.



SCHEME 3. Ionizations leading to competing products.

collected with an interfaced computer using Perkin–Elmer software. Rate constants were determined by nonlinear regression fitting of the uv–vis data to the integrated first-order rate equation using GraFit (Erithacus Software) on a MS-DOS computer (MS Windows 3.1) or by initial rate methods, as indicated.

Synthesis and product analysis. 2-(1-Hydroxybenzyl)thiamin, HBzT **(2)**, was prepared by condensation of benzaldehyde and thiamin (*2, 6, 7, 9*).

2-Benzoyl-5-(2-hydroxyethyl)-4-methylthiazole **(4)** was synthesized from HBzT as described by Oka (*7*) and by a multistep independent route which gave the same material (*10*). *Anal.* Calcd for $C_{13}H_{13}O_2NS$: C 63.14; H, 5.3; N, 5.66. Found (Galbraith Laboratories): C, 63.22; H, 5.55; N, 5.46. High-resolution mass spec-

trometry of parent ion: Calcd: 247.0667. Found: 247.0633. In addition to uv-vis spectra of products in kinetic runs, larger scale reactions were run in order to permit isolation and additional analysis. Products were isolated by extraction and column chromatography on silica gel with ethyl acetate/hexane eluant. Structures were determined by a combination of high-resolution mass spectrometry, ^{1}H NMR, and uv–vis spectra. Compound **3,** 2,5-dimethyl-4-amino-pyrimidine, was identified by spectroscopic and physical comparison with the material reported by Williams which is produced by reduction of the bisulfite cleavage product of thiamin (*6, 11*). 2-Benzoyl-5-(2-hydroxyethyl)-4-methylthiazole **(4)** had properties identical to those reported in the literature (*6*).

Buffer effects. Buffers were prepared with potassium chloride added to maintain constant ionic strength. After the products of the reactions were determined by following reactions to completion, kinetic studies were performed. At pH 7 and higher, first-order methods were used. Due to the very slow rate of reaction, initial rate methods were used below pH 7. We were able to monitor the appearance of products directly by uv-vis spectroscopy and did not use derivatives.

RESULTS

We determined rate constants at a number of points between pH 5 and 10. These values generally agree with those in the pH rate constant profile reported by Crane and Washabaugh (*2*) (a complete study is in progress). Repetitive uv–vis spectra throughout the course of the reactions permitted us to observe formation of expected and unexpected products. Above pH 8, the final spectra are those of combinations of benzaldehyde, thiamin, and the ring-opened pseudobase of thiamin (*5*). The rates, products, and absence of buffer catalysis in this region are consistent with all previous reports (*2, 9*) (Table 1).

In contrast to the expected patterns at high pH, uv–vis spectra of the products of reactions done below pH 8 indicate a pH-dependent change of products from benzaldehyde and thiamin to what were at first unidentified products. The relative amount of benzaldehyde and thiamin decreases compared to other products as pH is decreased. In particular, an intense new absorption develops, λ_{max} = 328 nm, along with peaks in the uv which do not correspond to those of thiamin or benzaldehyde. These spectra are identical to those reported by Oka and co-workers for the alternative decomposition route of HBzT in methanol containing triethylamine (*7*).

Preparative scale reactions were performed in order to isolate and identify products. Reactions at pH 9 and higher yield thiamin, benzaldehyde, and benzoin as principal products (identified by ^{1}H NMR and uv spectra). The products from the reaction at pH 6 were isolated and identified as those reported by Oka for the reaction in methanol (*7*): 2,5-dimethyl-4-amino-pyrimidine **(3)** and 2-benzoyl-5-(2-hydroxyethyl)-4-methylthiazole **(4)**. Compound **4** has a strong n to π^* band (λ_{max} = 328 nm; $\varepsilon \approx$ 10,000) where there is no significant absorbance due to HBzT, thiamin, or benzaldehyde.

The change in product distribution as a function of pH can be readily appreciated

TABLE 1

Effects of Buffers on Observed Rate Constants for the Decomposition of HBzT (to **3** and **4** or to Benzaldehyde and Thiamin)

T (°C)	pH	Buffer	Conc M	λ (nm)	k
40	5.2	KOAc	0.1, 0.33, 0.65, 1.0	328	$6.5 \pm 0.4 \times 10^{-7}$ s^{-1} (buffer independent)
25	9.5	K_2CO_3	0.02, 0.03, 0.04, 0.06	272	$1.2 \pm 0.1 \times 10^{-4}$, s^{-1} (buffer independent)
40	8.1	Tris	0.10	328	$3.20 \pm 0.4 \times 10^{-5}$, s^{-1}
			0.25		$4.30 \pm 0.04 \times 10^{-5}$, s^{-1}
			0.50		$5.30 \pm 0.04 \times 10^{-5}$, s^{-1}
					($k_b = 5.3 \pm 0.4 \times 10^{-5}$ M^{-1} s^{-1};
					$k_o = 2.8 \pm 0.1 \times 10^{-5}$ s^{-1})
40	7.7	KP_i	0.05	328	$1.20 \pm 0.03 \times 10^{-5}$, s^{-1}
			0.15		$1.40 \pm 0.03 \times 10^{-5}$, s^{-1}
			0.30		$1.80 \pm 0.03 \times 10^{-5}$, s^{-1}
					($k_b = 2.4 \pm 0.2 \times 10^{-5}$, s^{-1}
					$k_o = 1.06 \pm 0.3 \times 10^{-5}$, s^{-1})

Note. Samples contained 2.5 mM HBzT and buffer. Potassium chloride was added to maintain ionic strength at 1.0. Product appearance was followed at the wavelength indicated by λ.

from a series of time scans at 328 nm at various pHs. (Fig. 1). The rate of formation of **3** and **4** from HBzT relative to thiamin and benzaldehyde increases with decreasing pH. Oka and co-workers established that HBzT (as the chloride hydrochloride) is converted to **3** and **4** in methanol containing triethylamine (reflux, 5 h) (*7*). They also observed that in alkaline methanol, benzaldehyde and thiamin are produced from HBzT (*7*).

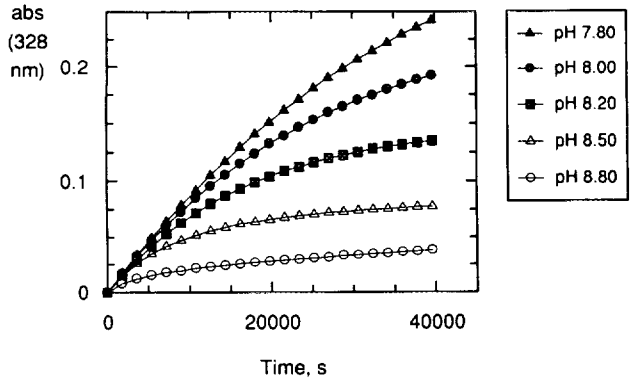


FIG. 1. Time scans at 328 nm (characteristic of formation of **4**) of the decomposition of HBzT at 40°C between pH 7.8 and 8.8 at a constant initial concentration of HBzT (10^{-4} M). The apparent first-order rate constant of the reaction increases with pH but the product changes from **3** and **4** to benzaldehyde and thiamin (which have a very low absorption at 328 nm). The thiamin produced in the reaction is converted to the pseudobase which undergoes ring-opening, leading to biphasic kinetics (*5*).

Buffer catalysis. Variation of carbonate buffer concentration at pH 9.5 has no effect on the observed rate constant for the conversion of HBzT to thiamin and benzaldehyde (Table 1). Variation of the concentration of acetic acid:acetate buffer at pH 5 also had no effect on the rate (Table 1). However, at pH 8 and 7, increasing concentrations of phosphate and Tris buffer increase the rate of HBzT's fragmentation to **3** and **4** (Table 1). The increase in the observed buffer rate constant with pH (for Tris) indicates that the reaction is general base-catalyzed, which can be formulated as a specific base-general acid-catalyzed process. The values of the buffer rate constants are comparable to those extrapolated from Crane and Washabaugh's Brönsted plots (*2*). A detailed study is currently in progress.

DISCUSSION

The decomposition of HBzT produces benzaldehyde and thiamin only at high pH. A competitive route, which is favored when a significant amount of HBzT is present as the protonated pyrimidine (pK_a = 5.3 (*2*)), gives the alternative set of products (**3** and **4**).

Rate laws of pH dependence. A minimal kinetic scheme that accounts for our observations is shown in Scheme 3. For the purposes of our general analysis, ionization constants (K_a, K_1, K_1, K_a') are set independent of the protonation state of other substituents ($K_1 = K_1'$ and $K_a = K_a'$). The actual situation is certain to involve differing ionization constants for the variously protonated species and will be subject to refinement with further studies. Since exchange of the C2α proton in deuterium oxide is faster than decomposition of HBzT (*9*), the removal of the C2α proton is treated as an equilibrium (K_1) preceding the rate-determining step.

HBzT **(SH)** is reversibly protonated at N_1' of the pyrimidine ring (**SH_2**), pK_a = 5.3 (*2*). Both **SH** and **SH_2** can transfer the benzyl carbon proton (K_1) to hydroxide to produce the conjugate base (**SH'** and **S**) which is an enamine (*12, 13*). The N_1'-protonated species **(SH')** undergoes rate-determining decomposition (k_2) to give **3** and **4**. To account for the lack of buffer catalysis at pH 5, where the pyrimidine ring is protonated, we suggest that the adjacent enolic proton serves as an intramolecular catalyst for the fragmentation (k_2) and that the protonated form of pyrimidine cleaves much more readily than the unprotonated form (Scheme 3). The internal enol is capable of functioning as an acid (The pK_a for the enol of mandelic acid is estimated to be 7.4 (*14*)) and has the potential for a high effective molarity).

Fragmentation of HBzT to **3** and **4** (k_2) requires initial loss of the C2α proton. Since the pK_a for loss of the proton from the benzyl carbon of HBzT is 15 (*13*) or higher (*15*) and hydroxide concentration is low, the concentrations of **SH'** and **S** will be very small compared to those of **SH_2** and **SH.** Formation of the alkoxide derived from the benzyl alcohol has a pK_a of 10.7 (*2*), and therefore the concentration of alkoxide is insignificant at pHs where **3** and **4** are produced. Thus

$$v = k_{obs}[\text{HBzt}] = k_{obs}([\mathbf{SH_2}] + [\mathbf{SH}]) = k_2([\mathbf{SH'}]) \qquad [1]$$

$$K_1 = [\mathbf{SH}']/[OH^-][\mathbf{SH}_2] \quad [2]$$

$$K_a = [\mathbf{SH}][H^+]/[\mathbf{SH}_2] \quad [3]$$

$$k_{obs} = K_1k_2[OH^-]/(1 + K_a/[H^+]). \quad [4]$$

Where the pyrimidine is mostly in protonated form,

$$[H^+] \gg K_a \text{ and}$$

$$k_{obs} = K_1k_2[OH^-], \quad [5]$$

giving the observed increase in rate with pH.

At higher pH, where the pyrimidine is not protonated;

$$k_{obs} = k_2K_1K_w/K_a. \quad [6]$$

K_w is the ion product of water and the contribution from this route to k_{obs} is pH-independent. In the presence of buffers, an extra term is needed for general base catalysis (k_2') in the conversion of **SH** to **3** and **4**.

In more basic solutions, the route to benzaldehyde and thiamin becomes significant as the concentration of pyrimidine-protonated material becomes negligible. The proportion of $\mathbf{SH}_2$ and **SH'** relative to **SH** and **S** decreases so that the route via k_2 becomes pH-independent, while that via k_4 increases in rate. The concentrations of **S** and **SH'** remain small compared to their conjugate carbon acids, and the concentration of $\mathbf{SH}_2$ is small compared to that of **SH**.

$$v = k_{obs}[HBzT] = k_{obs}([\mathbf{SH}] + [\mathbf{S}']) = k_4[\mathbf{S}'] \quad [7]$$

$$K_3 = [\mathbf{S}']/[\mathbf{SH}][OH^-] \quad [8]$$

$$k_{obs} = K_3k_4[OH^-]/(1 + K_3[OH^-]) \quad [9]$$

Where the hydroxyl group is not significantly dissociated,

$$K_3[OH^-] \ll 1 \text{ and } k_{obs} = K_3k_4[OH^-]. \quad [10]$$

This process becomes significant because its rate increases with pH while the fragmentation to **3** and **4** is pH-independent under these conditions.

At higher pH, where the hydroxyl becomes dissociated, $K_3[OH^-] \gg 1$ and $k_{obs} = k_4$. The profile reaches the final plateau above pH 10. There is no buffer-dependent term in the rate law for this region, consistent with the lack of catalysis by pH 9.5 carbonate buffer. In general, our data coincide with the curve generated from the equation used by Crane and Washabaugh to fit their data (*2*) in which the rate is the sum of two hydroxide-dependent rates and two "water" rates related by the pK for protonation of the pyrimidine. Thus, for the N_1-protonated species, with pK_a = 5.3,

$$k = k_0 + k_{OH}(1 + K_a/H^+).$$

As the pyrimidine is titrated the rate flattens to another water rate followed by an increase due to a hydroxide-dependent term

$$k = k_0' + k_{OH}'((H^+ + K_a)/K_a).$$

Buffer-dependent term. The buffer catalysis we observe at pH 8 adds a term which can be accommodated by steps associated with k_2' in Scheme 3. The details of this process are the subject of continuing investigations. If the fragmentation competes with conversion of the enol to the corresponding ketone, the buffer may be involved in promoting the fragmentation of ketone.

Effect on exchange studies. Our results also clarify some of the unexplained observations reported by Sable and co-workers (*9*). They used ^{1}H NMR to measure H–D exchange reactions of the C2α proton of HBzT. The equilibrium shown as K_1 in Scheme 3 in D_2O will lead to incorporation of a deuterium into the benzylic position. The decomposition products, **3** and **4**, should have been observed after an extended period of reaction. However, we find that in this case ^{1}H NMR will not readily distinguish these from what were the expected products, thiamin and benzaldehyde. The peaks assigned by Mieyal *et al.* to benzaldehyde (where the benzal proton has been exchanged for a deuteron) and thiamin are generally coincidental with those in **3** and **4**. Thus, observation of exchange at C2α is correct but the assumed products of the subsequent fragmentation are not. This does not necessitate any changes in conclusions about the rate of exchange.

Points to consider. General acid-base catalysis is unlikely to be involved in the conversion of HBzT to thiamin and benzaldehyde. The critical assumption that benzaldehyde and thiamin form below pH 8 (*2*, *16*) is contrary to our observation that **3** and **4** are the exclusive products under those conditions. Our preliminary studies indicate that only in the pH-independent process of formation of **3** and **4** from the unprotonated form of HBzT is the reaction subject to buffer catalysis. This is likely to be the conventional removal of a proton from a carbon adjacent to a carbonyl group. At high pH, where the reaction is proportional to hydroxide concentration, thiamin and benzaldehyde are the products and there is no buffer catalysis. Crane and Washabaugh observed buffer catalysis only at the lower pH (*2*).

Generalizations. The Oka fragmentation of C2-carbinyl derivatives of the conjugate acid of thiamin (*6*, *7*) should occur only in adducts of aldehydes. The key step in the reaction sequence is the conversion of the enamine formed by loss of the C2α proton. Adducts derived from ketones, such as those formed from benzoin (*1*), acetoin (*17*), acetolactate (*18*), or pyruvate (*5*), have no C2α proton; the conjugate base cannot form and fragment. Alkyl ethers of the C2α hydroxyl (used in proton transfer studies) also cannot fragment (*12*, *13*).

The adduct of benzaldehyde and thiamin diphosphate has been proposed as an intermediate in the action of benzoylformate decarboxylase (*19*). If fragmentation results from transfer of the enolic proton to the methylene bridge of HBzT, then the fragmentation of the diphosphate analogue of HBzT can be avoided by the enzyme controlling the conformation of the adduct. If the enol proton is held remote from the bridge, fragmentation is blocked. On the other hand, appropriate orbital alignment can lead to formation of thiamin diphosphate and benzaldehyde as has been discussed for the case of thiamin and pyruvate (*20*).

Conclusions. The decomposition of HBzT in aqueous solution occurs by pathways under neutral and acidic conditions which lead to fragmentation reactions resulting from ionization of the carbon acid derived from the protonated pyrimidine

of HBzT. It is proposed that the fragmentation involves intramolecular general acid catalysis. The decomposition of HBzT to thiamin and benzaldehyde is not subject to buffer catalysis. Thus, the special mechanisms proposed to explain catalysis by Brönsted acids in the decomposition of the benzyl alkoxide derived from HBzT to thiamin and benzaldehyde (and for related compounds) should be reconsidered.

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