JOURNAL OF PHYSICAL ORGANIC CHEMISTRY
J. Phys. Org. Chem. 2004; **17:** 507–510
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/poc.764

Fast fragmentation and slow protonation: a buffer-dependent isotope effect in reactions of *N*-methyl hydroxy(benzylthiamine) analyzed by the Keeffe–Jencks equations†

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Received 24 June 2003; revised 22 September 2003; accepted 22 September 2003

ABSTRACT: The fragmentation of 2-(1-hydroxybenzyl)thiamine in neutral solution (to cleave the pyrimidine and thiazolium) has been shown to compete very effectively with elimination of benzaldehyde to produce thiamine in neutral solution. The fragmentation is believed to involve protonation competing with C—N bond cleavage in the C2α conjugate base as a rate-determining step. We report that proton removal from C2α of *N1′*-methyl-2-(1-hydroxybenzyl)thiamine (MHBnT) is rate-limiting in low concentrations of pH 6 phosphate buffer: reprotonation competes with the subsequent fragmentation step (cleaving the pyrimidine–thiazolium bridge derived from thiamine) at higher buffer concentrations. Comparison of the observed rates of reaction of protio and C2α-deutero MHBnT reveals a non-linear variation of the kinetic isotope effect that fits precisely to a ratio derived from the Keeffe–Jencks rate law formulation for $E1_{CB}$ reactions. The fragmentation step is clearly distinct from the proton removal step and the isotope sensitivity is limited to the initial step. The variation of the isotope effect is a result of changes due to differing contributions from the hydroxide and buffer-catalyzed reaction mechanisms. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: fragmentation; protonation; isotope effect; thiamine; Keeffe–Jencks equations

INTRODUCTION

The base-catalyzed addition of thiamine to benzaldehdye produces 2-(1-hydroxybenzyl)thiamine (HBnT), an intermediate in the thiamine-catalyzed benzoin condensation. The reaction requires loss of the proton from C2 of the thiazolium ring, generating the ylide.[1,2] This elusive species was definitively studied by Washabaugh and Jencks.[3–5] In the benzoin condensation, the C2α proton of HBnT is transferred to a base, with the resulting anion adding to the carbonyl carbon of a second benzaldehyde. In alkaline solution (pH > 9) in the absence of benzaldehyde, HBnT reverts to thiamine and benzaldehyde. However, in neutral and acidic solutions HBnT does not revert to thiamine and benzaldehyde but instead fragments to the alternative set of products, dimethylaminopyrimidine (DMAP) and phenyl thiazole ketone (PTK)[6] (Scheme 1).

The reaction is catalyzed by hydroxide and other anionic Brønsted bases, promoting the loss of the proton from C2α. At pH 7, the rate of conversion of HBnT to the fragmentation products is about 1000 times greater than the rate of formation of thiamine and benzaldehyde. The proportion of fragmentation relative to elimination reflects the extent to which HBnT is protonated at *N1′*.[7] Jencks noted that such a result does not *require* that the kinetically active species be that which involves the thermodynamic site of protonation. He suggests that producing a compound with an alkyl group in place of the proton can simplify the problem: 'Model Compounds. The problem with the proton is that it is mobile, and its position on one or another atom in the transition state cannot be decided from the rate law of a reaction. Now if one substitutes a methyl group for the proton, the position of a group which differs only slightly from the proton in its polar charge is known and from the behavior of the model compound the behavior of the corresponding protonic compound may be inferred.'[8] Thus, the *N1′* methylpyrimidinium species (MHBnT), prepared from HBnT and dimethyl sulfate, fragments without competition from the elimination reaction (even in alkaline solutions where HBnT releases benzaldehyde). The rate is consistent with that observed for the protonated species.[7] The *N1′*-benzylpyrimidinium compound (BHBnT) also follows the same reaction patterns.[9]

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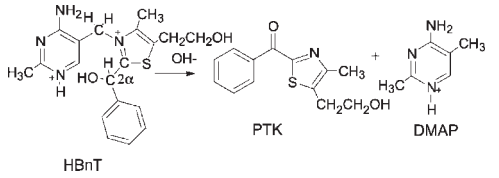
Contract/grant sponsor: Natural Sciences and Engineering Council of Canada.
†Selected paper part of a special issue entitled 'Biological Applications of Physical Organic Chemistry dedicated to Prof. William P. Jencks'.



Scheme 1

Extensive kinetic analysis of phosphate-catalyzed fragmentation of HBnT and its alkylated analogues showed that the rate-determining step in the fragmentation of protonated or alkylated HBnT changes from removal of the proton from C2α at low buffer concentrations towards fragmentation of the conjugate base at higher buffer concentrations.[10] The resulting dependence of the observed rate on buffer concentration was analyzed by the methods used by Keeffe and Jencks for $E1_{CB}$ reactions.[11,12] This yielded the surprising result that the rate constant for the fragmentation step is very large, competitive with that for protonation of the carbanion (which can be drawn as an enamine). A mechanistic scheme that is consistent with the observed kinetics is shown in Scheme 2.

This mechanism requires that there be a primary (H/D) kinetic isotope effect under all conditions. Thus, we have investigated the kinetic isotope effect as a function of buffer concentration in the fragmentation of MHBnT and have found that the results are consistent with this expectation.

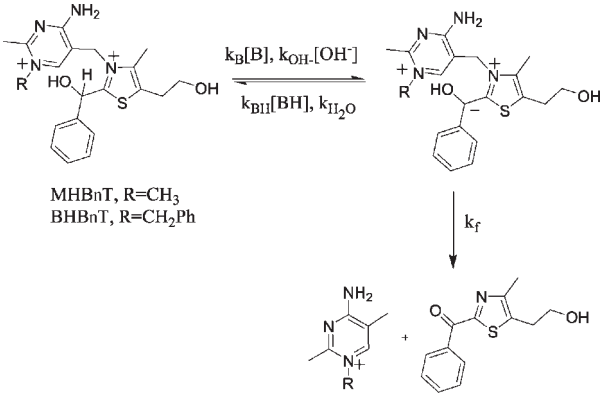
EXPERIMENTAL

Potassium phosphate buffer solutions were kept in a jacketed beaker maintained at 40 °C. A pH electrode was standardized against reference solutions at the same temperature. Ionic strength was maintained at 1.0 by the addition of potassium chloride. The dependence of observed rates on buffer concentration was measured in solutions at pH 6.1, where $[H_2PO_4^-] = 2[HPO_4^{2-}]$.

Synthesis of MHBnT and C2α-deutero-MHBnT

HBnT was synthesized by the procedure of Doughty *et al.*[13] followed by methylation by dimethyl sulfate according to the method described by Zoltewicz.[14] MHBnT was recovered as a white solid in 18% overall yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.3 (s, 1H), 8.5 (s, 1H), 7.7 (s, 1H), 7.4–7.3 (m, 5H), 6.8 (s, 1H), 6.3 (s, 1H), 5.3 (s, 2H), 3.8 (m, 2H), 3.5 (s, 3H), 3.1 (m, 2H), 2.5 (s, 3H), 2.3 (s, 3H). ESI-MS (high resolution) $[C_{20}H_{25}N_4O_2S]^{2+}$: calcd 385.1692; found 385.1679.

For 2α-deutero-MHBnT, benzaldehyde-1-*d* was used with a similar work-up substituting deuterium chloride and deuterium oxide in place of hydrogen chloride and water. The incorporation of deuterium was confirmed by the lack of the C2α proton signal in the proton NMR spectrum and the exact mass and fragmentation pattern observed by electron ionization mass spectrometry. Overall yield 26%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.3 (s, 1H), 8.5 (s, 1H), 7.7 (s, 1H), 7.4–7.3 (m, 5H), 6.8 (s, 1H), 5.4 (s, 2H), 3.8 (m, 2H), 3.6 (s, 3H), 3.1 (m, 2H), 2.5 (s, 3H), 2.3 (s, 3H). ESI-MS (high resolution) $[C_{20}H_{24}DN_4O_2S]^{2+}$: calcd 386.1754; found 386.1755.



Scheme 2

Kinetic measurements

The fragmentation of MHBnT was followed by monitoring the increase in absorbance at 328 nm (λ_{max} for PTK). Data were collected on an interfaced computer and analyzed with a curve-fitting program. After establishing that the observed kinetics fit a first-order rate law, we used the method of initial rates to obtain additional rate data. In the case of the deuterated reactant, this is necessary because the isotope exchange competes with the overall reaction, converting the remaining deutero-MHBnT to the unlabeled form in direct competition with fragmentation. Reactions were followed to conversion of 2% of the reactant, transferred to vials and incubated at 40 °C in a water-bath. After 10 half-lives the final absorbance was measured to determine the total PTK that was formed (to provide accurate concentration measurements in each sample).

RESULTS

The variation in the rate of fragmentation of MHBnT in 0.01–0.4 M potassium phosphate buffer at pH 6.1 is concave downwards, consistent with a change in rate-determining step with increasing buffer concentration. Although the rate does not become independent of buffer concentration in this range, the change is sufficient to provide accurate data analysis to determine the rate constants and ratios that generate the curvature.

Application of the steady-state approximation to Scheme 2 gives the equation

$$k_{obs} = \frac{(k_B[B] + k_{OH}[OH^-])k_f}{k_{BH}[BH] + k_{H_2O} + k_f} \quad (1)$$

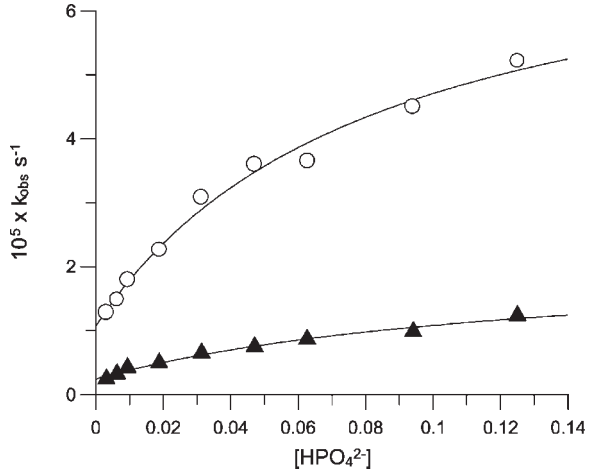


Figure 1. Dependence of the observed first-order rate coefficient on buffer concentration for MHBnT (○) and MHBnT-C2α-*d* (▲) at 40 °C, $I = 1.0$. Curves are fitted to the Keeffe–Jencks equation for a mechanism with a buffer-dependent change in rate-determining step

which describes a rectangular hyperbola. The data points in Fig. 1 are fitted to this equation, which is based on a mechanism with the rate-determining step changing from proton transfer to fragmentation with increasing buffer concentration. From this plot, the following values were obtained by fitting the data graphically according to the Keeffe–Jencks procedure:[12] $k_\infty = 7.7 \times 10^{-5}\,s^{-1}$ and $k_0 = 1.1 \times 10^{-5}\,s^{-1}$ for MHBnT; $k_\infty = 2.2 \times 10^{-5}\,s^{-1}$ and $k_0 = 2.4 \times 10^{-6}\,s^{-1}$ for MHBnT-C2α-*d*.

Further application allows the determination of important rate constants and ratios (Table 1) using the extrapolated values for k_0 ($= k_{obs}$ at $[B^-] = 0$) and k_∞ ($= k_{obs}$ where the rate is independent of buffer concentration).

The ratio of the curves generated from the data in Fig. 1 yields the kinetic isotope effect as a function of buffer concentration (Fig. 2). The ratio of the two rate equations for the protio- and deutero-substrates yields a function that describes the buffer dependence of the isotope effect. This relationship can be simplified by the assumption that both the second step (k_f) and reprotonation of the enamine (by water or buffer) are isotope-independent as they are subsequent to removal of the proton or deuteron. This leads to the equation

$$k_{obs} = \frac{k_B^H[B] + k_{OH}^H[OH^-]}{k_B^D[B] + k_{OH}^D[OH^-]} \qquad (2)$$

Table 1. Derived rate constants and ratios for fragmentation of MHBnT (H/D) and BHBnT

Substrate	k_{B^-} ($M^{-1}\,s^{-1}$)	k_{BH}/k_f (M^{-1})
MHBnT	$(1.1 \pm 0.1) \times 10^{-3}$	6.6 ± 0.3
MHBnT-C2α-*d*	$(1.9 \pm 0.1) \times 10^{-4}$	4.1 ± 0.2
BHBnT	$(7.4 \pm 0.5) \times 10^{-3}$	56 ± 4

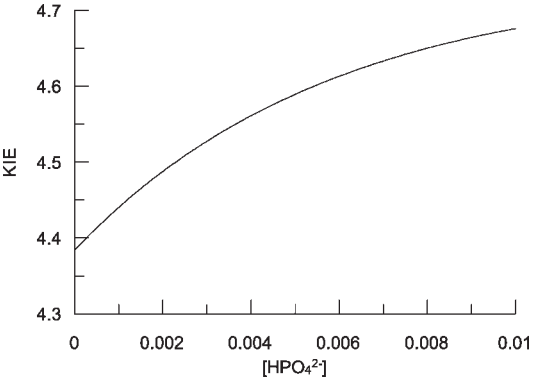


Figure 2. Observed KIE on fragmentation as a function of hydrogen phosphate buffer concentration

which describes the relationship between the data points in Fig. 2. From this relationship, it is apparent that the isotope effect is on the hydroxide-or buffer-catalyzed proton removal step (Table 2). At low buffer concentration, the isotope effect on proton removal due to hydroxide is observed. As buffer is increased, the isotope effect on buffer catalysis becomes the dominant contributor. Exchange-out of the deuterium from C2α in H_2O must be considerably faster than deuteration of the protio compound in D_2O. The solvent isotope effect on the overall fragmentation is inverse because the intermediate is more rapidly protonated in H_2O.

DISCUSSION

The values for k_{B^-} indicate that the rate of fragmentation is largely dependent on the basic component of the buffer. The fitted values that give k_{BH}/k_f establish that fragmentation competes with the kinetically significant rate of protonation of the C2α conjugate base. In the case of *N1′*-benzyl-2-(1-hydroxybenzyl)thiamine (BHBnT),[9] $k_{BH}/k_f = 56 \pm 4\,M^{-1}$. Therefore, fragmentation may compete more effectively with protonation in MHBnT than in BHBnT. This would cause saturation of buffer catalysis to require a higher effective concentration for MHBnT, as we report here. The result is consistent with protonation of MHBnT being slower than protonation of BHBnT, fragmentation being faster in MHBnT, or a combination of both.

The rate constant for buffer-catalyzed proton removal is about the same in the two compounds (based on k_{B^-}). If we make the reasonable assumption that the pK_a for

Table 2. Derived isotope effects on rate constants for the fragmentation of MHBnT (H/D)

Ratio (H/D)	Isotope effect
k_0	4.4
k_{B^-}	5.8

proton loss at C2α is independent of the group on N1′, then the rate constant for protonation of the intermediate is different in the two intermediates. Alternatively, the observation could result from the fragmentation step or steps being faster in MHBnT. At this point we must note the possibilities rather than choose among them.

Estimating the pK_a for the two compounds leads to the values of k_{BH} and k_f. We estimate the pK_a of the *N*′-alkylated HBnT to be 14.9. This is derived from the measured pK_a of 15.7 for the related methoxybenzyl methylthiazolium salt,[15] taking into account the inductive effect of the *N1*′-alkylated pyrimidine ring and the methoxy substituent at C2α.[16] MHBnT and BHBnT are likely to have similar pK_as for their respective C2α carbon acids as there is significant distance between *N1*′ and C2α. Assuming similar pK_as for both compounds gives $k_{BH} = 2.1 \times 10^6\ M^{-1}\ s^{-1}$ and $k_f = 3.8 \times 10^4\ s^{-1}$ for BHBnT versus $k_{BH} = 3.2 \times 10^5\ M^{-1}\ s^{-1}$ and $k_f = 4.8 \times 10^4\ s^{-1}$ for MHBnT. Therefore, it becomes apparent that the smaller k_{BH}/k_f ratio measured for MHBnT is probably a consequence of reprotonation being slower and fragmentation being slightly faster. Whereas k_{BH} differs by an order of magnitude, k_f is similar for both compounds. This suggests that the fragmentation step is not driven by interactions with the pyrimidine substituents. Thus, differences in k_{BH} would contribute a larger perturbation to the differing rate constant ratios (k_{BH}/k_f).

The KIEs observed in this study are large, consistent with proton removal being involved in the rate-limiting step at low buffer concentrations. The magnitude of the KIEs are in the range of Jordan and co-workers' reported values of 4–6 for the hydroxide-catalyzed removal of a proton from substituted 2-(1-methoxybenzyl)-3,4-dimethylthiazol-3-ium salts.[15,17] The theoretical maximum for a PKIE involving a carbon–hydrogen bond being broken in the rate-determining step at room temperature is ~ 7.[18] A more reactant-like or product-like transition state decreases this value. Therefore we conclude that within this buffer range, a carbon–hydrogen bond is being broken in the transition state of the rate-determining step, with the proton being centrally located between the Brønsted base and substrate. In the absence of buffer, the KIE of 4.4 involves rate-determining transfer of a proton or deuteron to hydroxide. An increase in the isotope effect is observed where the introduction of buffer begins to increase the partitioning between the deuterio and protio substrates. This is a consequence of the term due to buffer catalysis (k_{B^-}) being subject to a larger isotope effect.

The isotope effect on k_{B^-} is 5.8, approaching the upper limit for the conditions, whereas that on k_0 is only 4.4. A simple comparison of the magnitudes of the isotope effects on k_0 and k_{B^-} might indicate that the proton is more symmetrically positioned in the transition state of the buffer-catalyzed process. However, MHBnT has two localized positive charges that might affect the geometry of the encounter with the phosphate ion with its multiple negative charges. The solvation of hydroxide will also be very different so that the isotope effect will need to be determined in more examples in order to establish if these comparisons are general.

Decarboxylation of the conjugate of thiamine diphosphate and benzoylformate in the mechanism of benzoylformate-decarboxylase generates the conjugate base at C2α of the diphosphate of HBnT. The enzyme appears to function without fragmentation of the cofactor. Our results show that fragmentation competes very effectively with protonation. Since the decarboxylation should lead to an intermediate that is relatively long-lived, the enzyme does not rely on the addition of a proton from a Brønsted acid to avoid the destructive reaction.[19,20]

Acknowledgments

We thank the Natural Sciences and Engineering Council of Canada for support through a Discovery Grant. This paper is dedicated to Dr Bill Jencks, an inspiring and brilliant scientist. By following his rules we discover the questions as well as the answers.

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