Thiamin diphosphate (ThDP)-dependent decarboxylations are usually assumed to proceed by a series of covalent intermediates, the first one being the C2-thiazolium adduct with pyruvate, C2-α-lactylthiamin diphosphate (LThDP). Herein is addressed whether such an intermediate is kinetically competent with the enzymatic turnover numbers. In model studies it is shown that the first-order rate constant for decarboxylation cannot indeed exceed 50 s⁻¹ in tetrahydrofuran as solvent, ~10⁸ times faster than achieved in previous model systems. When racemic LThDP was exposed to the E91D yeast pyruvate decarboxylase variant, or to the E1 subunit of the pyruvate dehydrogenase complex (PDHc-E1) from *Escherichia coli*, it was partitioned between reversion to pyruvate and decarboxylation. Under steady-state conditions, the rate of these reactions is severely limited by the release of ThDP from the enzyme. Under pre-steady-state conditions, the rate constant for decarboxylation on exposure of LThDP to the E1 subunit of the pyruvate dehydrogenase complex was 0.4 s⁻¹, still more than a 100-fold slower than the turnover number. Because these experiments include binding, decarboxylation, and oxidation (for detection purposes), this is a lower limit on the rate constant for decarboxylation. The reasons for this slow reaction most likely include a slow conformational change of the free LThDP to the V conformation enforced by the enzyme. Between the results from model studies and those from the two enzymes, it is proposed that LThDP is indeed on the decarboxylation pathway of the two enzymes studied, and once LThDP is bound the protein needs to provide little assistance other than a low polarity environment.

According to the widely accepted mechanism of thiamin diphosphate (ThDP) I-dependent decarboxylations of α-ketoacids, there are a number of covalent intermediates formed between ThDP and substrate (C2-α-lactylThDP or LThDP), and ThDP and product (C2-α-hydroxethyl-ThDP or HEThDP), as exemplified with the reaction catalyzed by the enzyme pyruvate decarboxylase in Scheme 1 (1–5). The existence of LThDP on enzymes has received support from two sources recently. (a) Tittmann and coworkers (6) showed that LThDP can be trapped with acid quench after rapid mixing of pyruvate decarboxylase with substrate, and (b) the analogue of LThDP with a non-cleaveable C2-α-P bond phosphonolactyl-ThDP (PLThDP) was found to bind to both yeast pyruvate decarboxylase (YPDC, Ref. 7) and to the pyruvate dehydrogenase-E1 subunit (PDHc-E1, Ref. 8). The analogue PLThDP was also shown to be a reasonably strong inhibitor of the PDHc-E1 and could be co-crystallized with PDHc-E1, and displays behavior expected of an intermediate analogue.2 Yet, an earlier study by Kluger et al. (10) reported no detectable decarboxylation of LThDP when exposed to pyruvate decarboxylase.

In a pioneering chemical model reported by Lienhard and co-workers (11, 12), it was demonstrated that C2-α-lactylthiazolium (LThz) salts undergo decarboxylation rather fast, and the rate of decarboxylation is dramatically accelerated by solvents of lower dielectric constant, such as ethanol. That C–C bond cleavage is rate-limiting in this reaction was confirmed by 13C/12C kinetic isotope effect studies (13). Kluger (2) subsequently described in detail the pH dependence of the rate of decarboxylation of C2-α-lactylthiamin (LTh), showing that the rate is faster in the presence of the 4′-aminopyrimidine ring of the coenzyme. The unimolecular decarboxylation rate constants for these model reactions were considerably smaller than the value of the turnover numbers for such enzymes; the latter have typical values of ~60 s⁻¹/active site.

In this paper we address two issues related to the existence of this putative LThDP covalent intermediate. (a) We extended the model studies to solvents of lower dielectric constants and could achieve first-order decarboxylation rate constants as large as the enzymatic turnover numbers, and (b) we synthesized racemic LThDP and reconstituted both YPDC and PDHc-E1 with this putative intermediate. Although slower than the turnover numbers found when starting with pyruvate and enzyme-bound ThDP, the results clearly indicated that both enzymes can indeed utilize the synthetic material as substrate and, with a knowledge of the structures now available, suggested reasons why kinetic competence may be difficult to demonstrate. The results presented are especially timely in view of an unusual and unexpected ThDP-derived structure reported recently on a related enzyme, acetolactate synthase (14), on the basis of which the mechanism as drawn in Scheme 1 was challenged. Our results suggested that there is no need to invoke alternatives to the accepted mechanism; nucleophilic addition at the carbonyl carbon atom leading to formation of

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1 The abbreviations used are: ThDP, thiamin diphosphate; HEThDP, C2-α-hydroxethylThDP; LTh, C2-α-lactylthiamin; LThz, C2-α-lactyl-3,4,5-trimethylthiazolium; YPDC, yeast pyruvate decarboxylase; PLThDP, phosphonolactyl-ThDP; PDHc, pyruvate dehydrogenase complex; PDHc-E1, the first ThDP-dependent subunit of PDHc; DCIPP, 2,6-dichlorophenoinidophenol; THF, tetrahydrofuran; HPLC, high performance liquid chromatography.

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2 P. Arjunan, A. Brunskill, W. Furey, M. Sax, N. Nemeria, S. Zhang, and F. Jordan, manuscript in preparation.
the tetrahedral intermediate LThDP, which then undergoes decarboxylation, adequately accounts for the observations in a vast literature on the subject.

**EXPERIMENTAL PROCEDURES**

**Instrumentation**

Stopped-flow experiments were carried out on an Applied Photophysics SM.18MV instrument. UV-visible spectra and kinetics were carried out on a Varian (Cary 300) or PerkinElmer (Lambda 28) double-beam spectrophotometer.

**Synthesis**

**C2-α-Lactyl-4,5-dimethylthiazole t-butyl ester**

Under N₂ protection, n-BuLi (2.5 m solution, 8.5 ml, 21.3 mmol) was added dropwise to a solution of 4,5-dimethylthiazole (2 g, 17.7 mmol) in 20 ml of dry THF at −78 °C. The mixture was stirred for 30 min at −78 °C and t-butyl pyruvate (3.1g, 21.5 mmol) was added rapidly. The mixture was stirred for another 30 min at −78 °C. The aceton-dry ice bath was then removed, and the reaction mixture was allowed to warm to room temperature. Concentrated HCl (2 ml) was added to quench the reaction, followed by water (50 ml). The product was extracted with CH₂Cl₂ (25 ml, 3x). The combined organic layers were dried over Na₂SO₄. After evaporation of the solvent in vacuo, the crude product was chromatographed on silica gel (100 g) and eluted with ethyl acetate-hexane (1:4). The product was then used for the kinetic experiments without further purification.

**C2-α-Lactyl-3,4,5-trimethylthiazolium Triflate t-buty l Et yer**

Methyl triflate (0.7 ml, 6.2 mmol) was added to a solution of C2-α-lactyl-4,5-dimethylthiazole t-butyl ester (0.8g, 3.1 mmol) in 50 ml CH₂Cl₂. The mixture was stirred for 30 min. The crude product was obtained by removing the solvent in vacuo. The crude product was then chromatographed on silica gel (100 g) and eluted with MeOH-CH₂Cl₂ (1:20) providing 1.15 g of product (88%).1H NMR (500 MHz, CDCl₃/CDCl₄, δ, ppm): 4.09 (s, 3H, N₃–CH₃), 2.51 (s, 3H, C₃–CH₃), 2.46 (s, 3H, C₅–CH₃), 2.02 (s, 3H, C₃–CH₃), 1.50 (s, 3H, N₃–CH₃), 1.46 (s, 9H, −O–C(CH₃)₃).

**LThz Trifloromethanesulfonate**

Under N₂ protection, 0.5 g of C2-α-lactyl-3,4,5-trimethylthiazolium triflate t-butyl ester was dissolved in 0.5 ml of CF₃COOH. The solution was stirred for 30 min and dried in vacuo. The NMR spectrum shows no starting material left, and the product is pure. 1H NMR (500 MHz, CDCl₃, δ, ppm): 4.04 (s, 3H, N₃–CH₃), 2.50 (s, 3H, C₄–CH₃), 2.43 (s, 3H, C₅–CH₃), 2.06 (s, 3H, C₃–CH₃). The product was then used for the kinetic experiments without further purification.

**C2-α-LThDP t-butyl ester**

was synthesized by following the published procedure (10). It was then purified by HPLC (Altelt, Prophere C18 300 10μ, inner diameter 22 mm, length 250 mm) by eluting with dilute HCl (pH 2.5), followed by a mixture of water and MeOH (0–100% gradient). C2-α-LThDP was synthesized by following published procedure (10)

**Kinetic Studies**

**Decarboxylation of Model Compounds**—The reaction was monitored by stopped-flow by coupling the production of the enamine (decarboxylation) to its oxidation by 2,6-dichlorophenoindophenol (DCPIP, 18). The reaction was initiated by the addition of 2.5 mM LThDP in 20 mM potassium pH 7.0 buffer (20 mM, pH 7.0) was placed in a 1-ml quartz cell. The Tris-HCl buffer served as the reference. The reaction was initiated by the addition of coenzyme A to a final concentration of 0.1 mM.

**Monitoring the Release of Pyruvate from PDHc-E1-bound ThDP**—The depletion of NADH was monitored at 340 nm and 30 °C, coupling the conversion of pyruvate to lactate via lactate dehydrogenase and NADH. A solution (1-mL total volume) of 0.2 mM NADH, 10 units/ml lactate dehydrogenase, and 2 mM MgCl₂ solution of apo-E91D YPDC (0.5 mg/ml), 5 mM LThDP, 100 mM potassium Pi buffer (pH 7.0) was incubated at 25 °C. At various time intervals, 20-μl aliquots were removed, and the pyruvate released was measured using the NADH/lactate dehydrogenase coupled assay in the absence of pyruvate. The control contained every component except E91D apo-YPD C.

**Acetaldehyde Formed from LThDP by the Apo-enzymes of YPDC**—A solution of apo-E91D YPDC (0.5 mg/ml), 5 mM LThDP, 100 mM potassium Pi, 2 mM MgCl₂ (pH 6.1) was incubated at 25 °C. At various time intervals, 20-μl aliquots were removed, and the acetaldehyde produced was measured using the NADH/lactate dehydrogenase coupled assay in the absence of pyruvate. The control contained every component except E91D apo-YPD C.

**Pyruvate Release from LThDP Catalyzed by the E91D Apo-YPD C**—A solution of apo-E91D YPDC (0.5 mg/ml), 5 mM LThDP, 100 mM potassium Pi, 2 mM MgCl₂ (pH 6.1) was incubated at 25 °C. At various time intervals, 20-μl aliquots were removed, and the acetaldehyde produced was measured using the NADH/lactate dehydrogenase coupled assay in the absence of pyruvate.
RESULTS

Synthesis of LThDP

The racemic LThDP was first synthesized by Kluger and Smyth (10) in 1981 by condensing ThDP with pyruvic acid t-butyl ester, followed by removal of the t-butyl group with TFA. They reported that the t-butyl ester of LThDP underwent decomposition during column chromatography, even on a reversed-phase HPLC column. Therefore, the final LThDP product they used for the enzymatic activity measurements contained a significant background of ThDP. Contamination of LThDP with ThDP interferes with the interaction of LThDP and the apo-enzyme, because the ThDP would compete for the active center. We developed methods to separate the t-butyl ester of LThDP from ThDP on a C18 reverse-phase HPLC column. The NMR spectrum of both, the t-butyl ester of LThDP and of LThDP itself, displayed no detectable ThDP.

Interactions of LThDP with Yeast Pyruvate Decarboxylase

Reconstitution of the Apo-E91D Variant of YPDC with LThDP—Previous research from our laboratory demonstrated that the E91D substitution of YPDC endows the enzyme with a very useful property, the ability to readily exchange its ThDP at the optimum pH of 6.0 (20). Experiments were designed to monitor the release of both acetaldehyde and pyruvate from LThDP, i.e. catalysis of both the forward and reverse reactions in Scheme 1. As shown in Fig. 1, the rate of reduction of acetaldehyde by NADH in the overall PDHc assay. The rate of production of acetaldehyde was next monitored from several active center variants with two substitutions, the E91D to assure that ThDP could be replaced (20), and a second active center substitution already known to have a dramatic effect on the steady-state rate constants (18, 22). As seen in Fig. 1, although the substitution E477Q/E91D still allows formation of acetaldehyde, with the E51D/E91D and D28N/E91D double substitutions, essentially no rate can be seen for acetaldehyde formation. We conclude that the residues Glu-51 and Asp-28 have important roles somewhere between the binding of LThDP and acetaldehyde release (i.e. one of the steps depicted as k3,k 4, and k5 in Scheme 1), whereas Glu-477 has a more modest role in these steps. At the same time, evidence is also clear for release of pyruvate from LThDP (Fig. 1, open circles).

Interactions of LThDP with PDHc or the PDHc-E1 Subunit

Based on the proposed mechanism, the fate of the LThDP after binding to the apo-enzyme could be either decarboxylation in the forward direction (equivalent to formation of enamine), or decomposition in the reverse direction, leading to pyruvate release. The enamine could be trapped by DCPIP (in an E1-specific assay) or oxidized by E2 and E3, eventually producing NADH in the overall PDHc assay. The release of pyruvate could be detected by coupling the reaction via the lactate dehydrogenase/NADH assay. The PDHc-E1 activities (expressed as turnover numbers) as deduced from reaction progress curves for the E1-specific assay, the PDHc overall assay, and for pyruvate release from LThDP, were 0.115, 0.185, and 0.075 s⁻¹, respectively (23), all very low and rather similar. Apparently, under the steady-state conditions employed in these experiments, the observed activity (the rate-limiting step) is the release of ThDP to regenerate the apo-enzyme.

A single turnover experiment was next carried out to study the rate of decarboxylation of LThDP by oxidative trapping of the putative enamine with DCPIP. The reaction progress curve of DCPIP reduction (Fig. 4) consists of two phases, the pre-steady-state phase (the first 5 s) and the steady-state phase (linear part between 10 and 50 s). The total change in absorbance during the initial burst is about 0.11 A units, corresponding to 7.1 μM DCPIP reduction and 7.1 μM of PDHc-E1 active center concentration. This value is in good agreement with the

Reconstitution of Other Apo-enzymes of YPDC with LThDP—

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enzyme concentration used in the assay, 0.75 mg/ml corresponding to 7.5 M concentration of active centers (molecular weight of PDHc-E1 dimer is 200,000 with two active centers). This result suggests that both active centers on the PDHc-E1 bind LThDP and catalyze the reaction. The data were fitted to a first-order decay followed by a steady-state rate. The rate constant of the first-order decay is 0.4 s⁻¹ (for each active center, 0.8 s⁻¹/E1 dimer). This rate constant is an apparent rate constant encompassing three consecutive steps, binding of LThDP to apo-E1 (including a conformational change of the LThDP from the “S” to the “V” conformation), decarboxylation of LThDP, and oxidation of the enamine by DCPIP. Thus, it is a lower limit of the decarboxylation rate constant for LThDP on PDHc-E1. The actual rate constant of this step could be much faster.

**Decarboxylation of Model Compounds in a Non-polar Environment**

It had been reported in 1970 (11, 12) that the decarboxylation of LThz is accelerated by transferring the reaction to a non-polar solvent. Previous studies on the decarboxylation of model compounds were performed in water and EtOH, and it was concluded that the rate in EtOH is 10⁴ times faster than in water. But, all of the rate constants obtained were quite small (the largest first-order decarboxylation rate constant previously reported is 3.63 × 10⁻² s⁻¹, Ref. 2) and far below the enzyme turnover number (for YPDC, the kcat value is ~60 s⁻¹/subunit).

We studied the decarboxylation of the LThz and LTh (Fig. 5)
in CH₃CN (ε = 36.6) and in THF (ε = 7.5). The rate of decarboxylation was monitored via the reduction of DCPIP at 600 nm according to Equation 1, trapping the enamine intermediate derived from compounds analogous to LThDP in Scheme 1. DCI (3 M in D₂O) was used to maintain the LTh and LThz in the conjugate acid [COOH] form to prevent the decarboxylation of LTh or LThz before the stopped-flow experiments (11, 12).

In Equation 1, the first step is proton transfer, and it is presumably much faster than the remaining steps when a suitable base is chosen. The rate constant k₁ is the unimolecular decarboxylation rate constant, and k₂[DCPIP] is the second-order rate constant for oxidation. The reactions were carried out at 0.5 mM DCPIP and 0.2 mM LThz or LTh in the conjugate acid [COOH] form to prevent the decarboxylation of LTh or LThz before the stopped-flow experiments (11, 12).

The results with the enzyme are more complex, but also quite informative. When starting with pyruvate, Tittmann et al. (6) did observe formation of some LThDP, yet in an early reconstitution experiment using LThDP and apo-pyruvate decarboxylase no activity could be detected (10). Armed with the E91D YPDC variant, which can readily exchange its ThDP for LThDP, it can be argued that the enzyme can readily exchange its ThDP for LThDP and pyruvate. Almost certainly, under steady-state conditions this is because of the rate-limiting loss of ThDP from the enzyme. Nevertheless, the evidence was clear that LThDP was being partitioned on YPDC, reverting to pyruvate on the one hand,
and undergoing decarboxylation in the forward direction. With the PDHc-E1, under steady-state conditions both the reversal to pyruvate and decarboxylation were in evidence. In this case, we also undertook pre-steady-state experiments, in which we used DCCP to monitor the rate of decarboxylation. The rate constant in this case is of the order of 0.4 s⁻¹, still two orders of magnitude slow compared with the turnover number. There are several possible reasons for this, because the rate constant measures a composite of three steps, binding of LThDP, decarboxylation, and oxidation. The value determined in this experiment is indeed a lower limit. In the E1-specific assay using DCCP to trap the enamine, a PDHc-E1 activity of 0.385 units/mg, translating to a kₐₑₜ of 1.28 s⁻¹/E1 dimer or 0.64 s⁻¹/active site was detected (obtained by varying the DCCP concentration leading to this plateau value). Because the E1 activity is much higher according to the overall NADH assay, the rate-limiting step could be the oxidation of the enamine by DCCP, and the rate constant 0.64 s⁻¹ could be the upper limit for detection of enzyme-bound enamine with the DCCP assay. (Parenthetically, our results point to this important limitation of the E1-specific assay relying on DCCP when surveying variants of the E1 subunits, where the true kₐₑₜ and Kₑ values are often not reflected accurately.) The first-order rate constant of 0.4 s⁻¹ reports on the slowest step of the three steps, of which the rate-limiting step is most likely the binding of LThDP to E1. The rate constant for binding of ThDP to the E1 is ~10⁴ s⁻¹ M⁻¹, ~10⁴ times faster than of HEThDP,² so the binding of LThDP could indeed be 100 times slower than of ThDP. For an LThDP concentration of ~1 mm, the pseudo-first-order rate constant for binding could be as slow as 0.4 s⁻¹, the value determined by our experiments. In turn, the true rate constant for decarboxylation of LThDP on the PDHc-E1 could be much faster, as also found by Tittmann et al. (6) when reacting holo-pyruvate decarboxylases with pyruvate.

The rate constants reported in Table 1, for such an effect on both YPDC and PDHc-E1. We exploited the reaction depicted by the rate constants kₐₑₜ/kₐₑₜ in Scheme 1, with the hypothesis that should the enzyme push the equilibrium from HEThDP to the enamine, this would be consistent with such an enzymatic “solvent effect,” because a lower “effective dielectric constant” of the enzyme should favor the enamine over the HEThDP from the electrostatic view. In model studies, we showed that the pKₐ of HEThDP at the C₂α position is ~15 in Me₆SO-water mixtures and extrapolates to 18 in pure water (28), whereas PDHc-E1 can ionize it at pH 7 with a rate constant >1 s⁻¹ accounting for a rate acceleration of the kₐₑ_step of at least 10 million-fold by the enzyme.³ We had also shown that the C₂α-hydroxybenzyl analogue can be converted to the enamine on YPDC (29) by ionization at the C₂α position (pKₐ for this ionization in models is near 15; Ref. 30 and 31), and more recently we established this C₂α ionization of HEThDP on YPDC as well (32), with its even higher pKₐ quoted above. All of these results on the enzymes could be explained by an environmentally induced stabilization of the enamine intermediates. Our sole result regarding the effective dielectric constant on ThDP enzymes is derived from the fluorescence emission maximum of thiochrome diphosphate (a fluorescent ThDP analogue that is a competitive inhibitor of several ThDP enzymes) on YPDC, and its relationship to the same structure of phosphonolactyl thiamin, where the –COOH of the lactyl group is replaced by –PO(OCH₃)O₂H (replacing the scissile C₂α-COOH by an unreactive C₂α-P bond), a structure that clearly displays the S conformation characteristics. In contrast, in the crystal structure of PLThDP complexed to PDHc-E1, not only is the V coenzyme conformation found, but the C-2 atom and the N-4' atom are brought to within 3.5 Å or less of each other.² That the substituent at the C-2 atom induces steric hindrance is evidenced by the finding that both PLThDP, a stable LThDP analogue, and HEThDP, exist as their 1'-protonated form (7, 8). Hence, the bound conformation of LThDP must also be quite different from the conformation found in the free compound, and, the rate constant for this conformational change could be limiting the rate here detected. At the same time, as mentioned above, the rate of oxidation of the enamine by DCCP on enzymes is not a true measure of the rates of decarboxylation, because DCCP may have difficulty being recognized (i.e. gain access to) the active center, and this barrier could vary from enzyme to enzyme.

The dramatic solvent effect on the rate of decarboxylation in the two models reported here is explained by concentration of charges in going from the zwitterionic LThDP analogue ground state to the enamine-like transition state, with a much reduced charge separation. This observation suggests, but does not prove, that a similar “environmentally induced” rate acceleration also exists on the ThDP enzymes. In our laboratory, we have searched for such an effect on both YPDC and PDHc-E1. We exploited the reaction depicted by the rate constants kₐₑₜ/kₐₑₜ in Scheme 1, with the hypothesis that should the enzyme push the equilibrium from HEThDP to the enamine, this would be consistent with such an enzymatic “solvent effect,” because a lower “effective dielectric constant” of the enzyme should favor the enamine over the HEThDP from the electrostatic view. In model studies, we showed that the pKₐ of HEThDP at the C₂α position is ~15 in Me₆SO-water mixtures and extrapolates to 18 in pure water (28), whereas PDHc-E1 can ionize it at pH 7 with a rate constant >1 s⁻¹ accounting for a rate acceleration of the kₐₑ_step of at least 10 million-fold by the enzyme.³ We had also shown that the C₂α-hydroxybenzyl analogue can be converted to the enamine on YPDC (29) by ionization at the C₂α position (pKₐ for this ionization in models is near 15; Ref. 30 and 31), and more recently we established this C₂α ionization of HEThDP on YPDC as well (32), with its even higher pKₐ quoted above. All of these results on the enzymes could be explained by an environmentally induced stabilization of the enamine intermediates. Our sole result regarding the effective dielectric constant on ThDP enzymes is derived from the fluorescence emission maximum of thiochrome diphosphate (a fluorescent ThDP analogue that is a competitive inhibitor of several ThDP enzymes) on YPDC, and its relationship to the same structure of phosphonolactyl thiamin, where the –COOH of the lactyl group is replaced by –PO(OCH₃)O₂H (replacing the scissile C₂α-COOH by an unreactive C₂α-P bond), a structure that clearly displays the S conformation characteristics. In contrast, in the crystal structure of PLThDP complexed to PDHc-E1, not only is the V coenzyme conformation found, but the C-2 atom and the N-4' atom are brought to within 3.5 Å or less of each other.² That the substituent at the C-2 atom induc...
quantity measured in a series of 1-alkanols and water by Li and co-workers (29). The value recorded on YPDC fell between the values recorded in 1-pentanol and 1-hexanol interpolating to an effective dielectric constant near 13. Hence, in the enzyme reactions, once the LThDP is formed with the requisite V conformation, the barrier for the decarboxylation step can be significantly reduced by simply providing an apolar (non-aqueous) environment.

On the basis of our results here reported it is appropriate to ask whether the notion of a tricyclic thiamin, in which the amino nitrogen adds to C-2 of the thiazolium moiety and where LThDP is not on the reaction pathway of these enzymes, as suggested by Pang et al. (14) for the enzyme acetolactate synthase, is relevant to chemistry taking place on the enzymes. We wish to raise several points not raised in that paper that tend to argue against their hypothesis. (a) Regarding the reactivity of the C2-carbanion/ylide/carbene (all simply resonance contributions to the resonance hybrid), its structure had been established by Arduengo and colleagues (33); perhaps the most striking feature of their finding is that the 13C chemical shift of the thiazolium C-2 carbon is deshielded from 157 to 253 ppm on formation of the C2-carbanion/ylide/carbene. Subsequently, Brown (34) and Ikemoto* at Rutgers confirmed those results and showed that the species generated could lead to the expected products from either pyruvate or aldehydes. Chen et al. (35, 36) at Rutgers also showed that whether the C-2 conjugate base reacts as a carbanion or as a carbene, nucleophilic addition accounts for the reactivity, and insertion can be ruled out. (b) The model here reported for the decarboxylation step leads to first-order rate constants for LThDP analogues approaching (b)

(b) The model here reported for the decarboxylation step leads to first-order rate constants for LThDP analogues approaching (b)

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