Breslow Intermediates

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The Need for an Alternative to Radicals as the Cause of Fragmentation of a Thiamin-Derived Breslow Intermediate

*Michael Bielecki and Ronald Kluger**

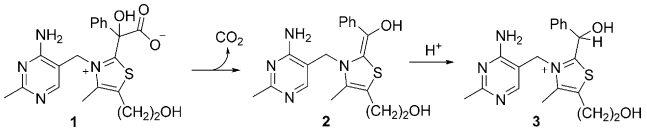
Abstract: *Mandelylthiamin (**1**) is a conjugate of benzoylformate and thiamin that loses CO_2 to form the classic Breslow intermediate (**2**), whose expected fate is formation of the thiamin conjugate of benzaldehyde (**3**). Surprisingly, it was observed that **2** decomposes to **4** and **5** and rearranges to **6** in competition with the expected protonation to give **3**. Recent reports propose that the alternatives to protonation arise from homolysis followed by radical-centered processes. It is now found, instead, that the spectroscopic observations cited in support of the proposed radical pathways are likely to be the result of other events. An alternative explanation is that ionization of the enolic hydroxy group of **2** and resultant electronic reorganization leads to C–C bond cleavage and non-radical intermediates that readily form **4**, **5**, and **6**.*

Breslow's insightful studies showed that the formation of acyl carbanion equivalents in decarboxylases result from addition of a nucleophilic carbene derived from enzyme-bound thiamin diphosphate to 2-ketoacids.[1] This led to generalizations in which the addition of thiamin-related N-heterocyclic carbenes (NHC) to aldehydes lead to synthetically useful acyl carbanion synthons.[2]

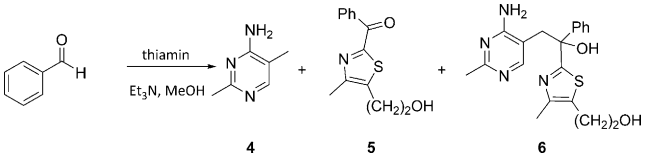
Mandelylthiamin (**1**, Scheme 1), the conjugate of thiamin and benzoylformate, loses CO_2 to produce the expected Breslow intermediate **2**. Protonation gives isolable 2α-(1-hydroxybenzyl)thiamin (**3**).[3]

An attempt by Oka to achieve a thiamin-catalyzed benzoin condensation of benzaldehyde via formation of **2** unexpectedly gave **4**, **5**, and **6** (Scheme 2).[4] Studies of the reactions of **2** revealed its spontaneous fragmentation to Oka's products **4** and **5** where the absorbance of **5** at 328 nm is characteristic, providing a spectroscopic handle for kinetic studies.[5]

McIntosh and co-workers recently reported that N-heterocyclic carbenes react with benzaldehyde to give products that parallel the outcomes in Scheme 2.[6] They ascribe the results to reactions of radicals from homolysis of the Breslow intermediate observed in EPR spectra and computations. They propose that radicals lead to the products from **1** in Scheme 2, both the rearrangement and fragmentation forming via a radical pair from homolysis of the Breslow intermediate (Scheme 3).[7] Recombination and disproportionation lead to both sets of products. Simulated EPR spectra of expected radicals are consistent with observed spectra and



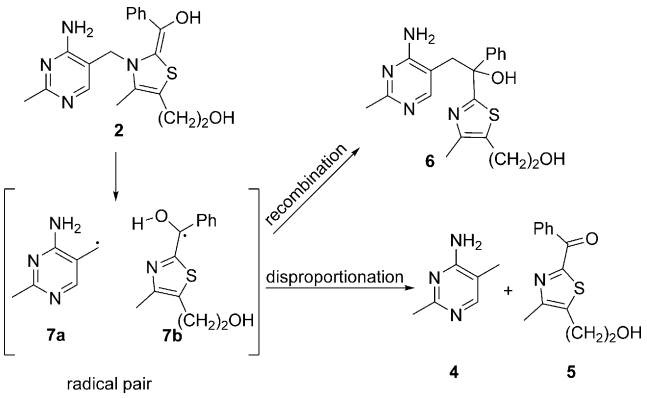
Scheme 1. Decarboxylation of **1** in neutral aqueous solutions produces Breslow intermediate **2** followed by protonation to the thiamin–benzaldehyde adduct **3**.



Scheme 2. Combining thiamin with benzaldehyde and triethylamine as base in refluxing methanol results in products **4**, **5**, and **6** via a fragmentation and rearrangement of **2**, respectively.

computations indicate that the proposed radicals are energetically accessible. Nonetheless, it is surprising to encounter formation of radicals under the conditions in which we see similar products from **2**.

McIntosh and co-workers generate their Breslow intermediate in methanol by combining benzaldehyde with the NHC precursor and DBU in the presence of oxygen. Under these conditions, other reactions can produce radicals: 1) air-oxidation of benzaldehyde;[8] 2) reaction of benzaldehyde with the Breslow intermediate;[9] 3) reaction of benzaldehyde with the conjugate acid of the intermediate;[9] 4) air-oxidation of the intermediate;[10] and 5) air-oxidation of benzoin.[11] These potential side reactions and lack of controls permit neither confident assignment of a signal to the proposed radical pair nor a basis for quantitation. Attempts to detect or



Scheme 3. A radical mechanism, based on McIntosh and co-workers' proposal, for the formation of **4**, **5**, and **6**. The Breslow intermediate **2** undergoes a spontaneous decomposition to a radical pair. The pair undergoes disproportionation to form **4** and **5** and recombination to give **6**.

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isolate TEMPO adducts that would be expected from radicals also gave negative results.[7]

It is well-established that **1** decarboxylates in neutral aqueous buffers to form **2**, which rapidly undergoes fragmentation to **4** and **5** (and potentially other products).[5] Thus, we prepared **1**[12] and analyzed the products of its decarboxylation in phosphate buffers in D_2O by ^1H NMR spectroscopy. We added genuine samples of potential products and compared their NMR signals with those from the reaction in solution.[13] We confirmed the formation of **3**, **4**, **5**, and **6** in relative molar concentrations of 100:30:30:3. We had not previously noted formation of **6**, as previous studies were concerned with the rate of the decarboxylation process. We also note the formation of small amounts (2–3 mol%) of thiamin and benzoic acid, which could result from hydrolysis of 2-benzoylthiamin upon oxidation of **2**.[14] However, in the absence of oxygen, the products do not include benzoic acid. In the presence of oxygen, benzaldehyde, produced from **3**, reacts with oxygen to form benzoic acid.

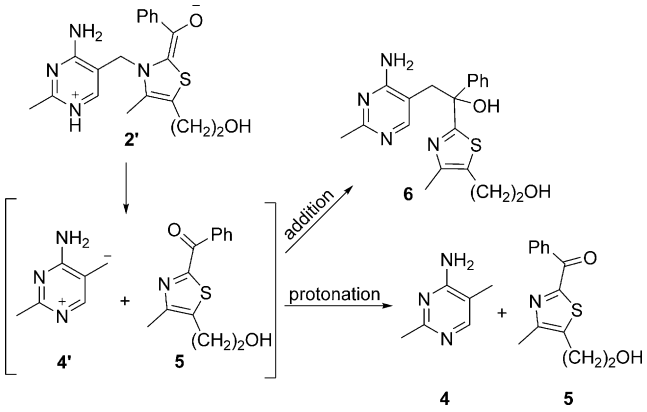
We attempted to trap intermediate **7a**, which would form from a radical pathway, by adding the water-soluble radical trap 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (4-hydroxy-TEMPO) to the reaction mixture. UV/Vis spectroscopy revealed formation of none of the characteristic band at 328 nm from **5**.[5b] Lower concentrations of 4-hydroxy-TEMPO significantly decrease that band. Analogous reactions were conducted in D_2O and studied by ^1H NMR spectroscopy. These reveal only two major products: thiamin and benzoic acid. This is consistent with oxidation of **2** by 4-hydroxy-TEMPO as reported by Studer and co-workers, who propose a single-electron transfer (SET): TEMPO or 4-hydroxy-TEMPO, oxidizes the Breslow intermediate to an acyl derivative in 2:1 stoichiometry, respectively.[15] The aminoxyl radical[16] itself is reduced to the corresponding hydroxylamine. In the present case, oxidation of the intermediate produces 2-benzoylthiamin, which reacts rapidly with water to produce thiamin and benzoic acid. Both the UV and ^1H NMR spectra indicate that fragmentation products do not form when the decarboxylation of **1** occurs in the presence of mM amounts of 4-hydroxyTEMPO. The rate constant for the fragmentation reaction of **2** at 25 °C is about $10^4\ s^{-1}$.[5a] Since we can detect the presence of fragmentation products at about 1 mol%, the rate constant for reaction of 4-hydroxy-TEMPO with **2** must be at least $10^6\ s^{-1}$. This suggests why McIntosh and co-workers do not isolate TEMPO-radical conjugates.[7]

We also investigated the fragmentation of **2** using nitrone spin traps and EPR. With *N*-tert-butyl-α-(2-sufophenyl)nitrone sodium salt (2-SPBN-Na) as a water-soluble spin trap, EPR established a limit of detection of 100 nM with 4-hydroxy-TEMPO standards. The samples contained 10 mM **1** and 20 mM 2-SPBN-Na in pH 7.0 phosphate buffer. All of the EPR experiments where conducted in the absence of oxygen and light. UV spectroscopy reveals that 10 mol% **1** undergoes fragmentation. We recorded the initial EPR spectrum within 20 min and another after 5 h and a reference spectrum in the absence of **1**. There is no EPR signal over 5 h. However, exposure of the reaction mixture after 5 h to oxygen produces a signal. The control did not produce a signal when exposed to oxygen.

We conducted analogous studies with 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO). As with 2-SPBN-Na, there is no EPR signal, while exposure to oxygen produces a signal. Given the oxidative reactivity of 4-hydroxy-TEMPO towards **2,** this results from reduction of aminoxyl radicals by **2** to the EPR-silent hydroxylamines. Exposure to oxygen regenerates the radicals.[17] By integrating the EPR signal with 1.0 μM 4-hydroxy-TEMPO solution as a standard, we estimate that 0.8 nmol of a radical species is trapped. Assuming fragmentation to be a radical process occurring at 10 mol%, this corresponds to only 0.05 mol% interception by the spin trap. This very low extent of trapping may arise from radical pair recombination being faster than desolvation or from minor side reactions. Nucleophilic additions to both nitroso and nitrone spin traps may lead to false-positive results.[18] Hydroxylamines that form from addition react with atmospheric oxygen to give EPR-active aminoxyl radicals. Based on the nucleophilic properties of Breslow intermediates, such an event can account for a signal, which is not relevant to the fragmentation process.[19] Finally, the small amount of benzaldehyde that forms would be air-oxidized and then produce radicals.

If a radical pair were responsible for the EPR signal, our spin-trapping results indicate that radical disproportionation and recombination would have to be faster than desolvation. From our product studies, the radical disproportionation/recombination ratio (k_{disp}/k_{rec}), from the relative amounts of **4** and **5** to **6,** would have to be about 10:1. Studies on resonance-stabilized radical pair recombination and disproportionation reactions show that spin delocalization greatly favors recombination over disproportionation. Typically, k_{disp}/k_{rec} is below 0.1.[20,21] Although steric factors may affect recombination, values of $k_{disp}/k_{rec} > 1$ are not accessible.[21] Both **7a** and **7b** exhibit delocalization of electron spin into aromatic rings. Moreover, the fragmentation, which is a β-elimination, would require homolysis of an RO–H bond, a process that is not normally accessible.[22] Therefore, in a radical process more of the rearrangement product (**6**) would form than would **4** and **5**. Under these circumstances, $k_{disp}/k_{rec} \approx 10$ is inconsistent with reactivity patterns of radical pairs.[20a]

Thus, decarboxylation of **1** in aqueous solutions produces **2** from which the rearrangement product **6** and fragmentation products **4** and **5** are clearly formed. Therefore, the reactivity of **2** parallels the reactivity of Breslow intermediates described by McIntosh and co-workers. Unlike generation of a Breslow intermediate from a mixture containing a thiamin-like carbene precursor, benzaldehyde, and base, decarboxylation of **1** avoids side reactions that lead to radicals. Our spin-trapping experiments produce only a weak EPR signal, corresponding to 0.05 mol% of the total amount of radicals that would form if fragmentation were a radical process. The rapid reduction of aminoxyl radicals by the Breslow intermediate poses an experimental challenge where oxygen would rescue the reduced spin-trap adducts. However, this will also lead to oxidation of hydroxylamines that would have been formed by nucleophilic additions to the spin trap, giving an irrelevant EPR signal. The product distribution is also inconsistent with a radical pair mechanism, where the recombination product (**6**) would exceed the fragmentation products (**4** and **5**).



Scheme 4. An anionic mechanism for the fragmentation and rearrangement reactions of the Breslow intermediate.

A more likely mechanism for fragmentation and rearrangement involves a reactive carbanion (**4′**; Scheme 4). We propose that enol **2** ionizes to form a zwitterion (**2′**) by transfer of the proton from its hydroxyl group substituent, followed by C–N bond-breaking. The product (**5**) derives stability from re-aromatization of the thiazole ring and drives elimination of a charge-stabilized carbanion. The ionization of the OH group of **2** closely resembles that of the enol form of mandelic acid with its trio of electronegative atom substituents acidifying the hydroxyl group with $pK_A = 6$.[23] The incorporation of a single deuterium into the CH_3 group of **4** upon fragmentation in D_2O is also consistent with the formation of the carbanion precursor and with the formation of **6** from the addition of **4′** to **5**.[24] D_2O cannot serve as a source of deuterium atoms in a radical mechanism, owing to the very high energy of hydroxyl radicals.[25]

In conclusion, our results indicate that the hypothetical formation of radicals does not account for the products of fragmentation from Breslow intermediate **2**. It is also important to note that enzymes produce Breslow intermediates that do not undergo fragmentation or rearrangement.[26] Yet, the potential fragmentation rate constant is larger than the enzymic k_{cat}. This is consistent with enzymes avoiding delocalization of the C2α carbanion as an incidental consequence in their evolved efficiency.[27]

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Conflict of interest

The authors declare no conflict of interest.

Keywords: Breslow intermediates · C–C bond cleavage · fragmentation · reaction mechanisms · thiamin

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Communications

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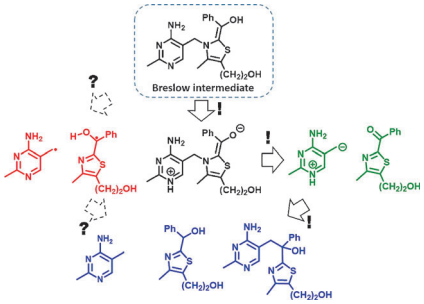

Perhaps not so radical: A Breslow intermediate surprisingly gives a mixture of products (blue). It has been proposed that these form via radicals (red), but it is now suggested that a non-radical process prevails (green).