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Solvent Polarity Effects and Limited Acid Catalysis in Rearrangements of Model Radicals for the Methylmalonyl-CoA Mutase- and Isobutyryl-CoA Mutase-Catalyzed Isomerization Reactions

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Coenzyme B_{12}-dependent enzymes catalyze a number of rearrangements via radical intermediates.[1] One of the better-studied enzymes in this group is methylmalonyl-CoA mutase (MCM), which catalyzes the interconversion of methylmalonyl-CoA with succinyl-CoA.[1,2] The closely related enzyme isobutyryl-CoA mutase (ICM) catalyzes a similar reaction, conversion of isobutyryl-CoA to butyryl-CoA.[3] The mechanisms of the radical rearrangement reactions are not known,[1] but a commonly assumed pathway involves 3-*exo* cyclization of radicals **1** to give intermediate cyclopropanoxyl radicals **2** that fragment to radicals **3** (Scheme 1). A small amount of rearrangement of a model for the methylmalonyl-CoA radical was found by Halpern,[4] but the reaction is not facile. Computations indicate that the reversible radical reaction in the MCM system is too slow for kinetic competence in the enzyme-catalyzed process with a barrier of ca. 24 kcal/mol for conversion of **3a** to **2a**.[5] The computational work suggested large reductions in the barriers for 3-*exo* cyclization reactions of carbonyl-protonated radicals, leading to the proposal that the rearrangements in the enzymes are catalyzed by "partial protonation" (hydrogen bonding to an acid) of the carbonyl group.[5,6]

Acid catalysis of the radical addition reaction in Scheme 1 not only offers a possible explanation for the radical reactions in the enzymes but also might be useful for organic synthesis. 3-*exo* Radical cyclizations onto cyclic ketones are known in the context of ring expansion reactions,[7] and limited kinetic information is available.[8] Acid catalysis and other polar effects on radical reactions are not well characterized, however, and no experimental data is available to evaluate the computational predictions for "partially protonated" radicals. We report here a study of models for the radicals formed in the MCM- and ICM-catalyzed rearrangements. Rate constants for reactions of neutral radicals are well estimated by computational methods, but reactions of acid-complexed radicals are much slower than predicted.

Radicals **5** (Scheme 2) are generated from precursors **4** by 266-nm irradiation in laser flash photolysis (LFP) studies or by reactions with stannyl radicals in preparative chain reaction sequences. Radicals **5** can rearrange via intermediates **6** to radicals **7**. The diphenylcyclopropyl reporter group in radicals **7** will rapidly fragment to give radicals **8** ($k \approx 5 \times 10^{11}$ s^{-1} at 20 °C),[9] thus serving as a trap for products **7** and providing a useful chromophore in LFP studies. In preparative reactions with Bu_3SnH, hydrogen atom transfer to **8** will give products **9**.

Reaction of the aldehyde radical **5a** gave the results shown in Figure 1A. The growing signal with $\lambda_{max} = 335$ nm is that expected for diphenylalkyl radical **8a**. The intensities of the maximum signals at $\lambda = 335$ nm were compared to the initial intensities of the signals at $\lambda = 490$ nm from the phenylselenyl radical to obtain yields; the yield of **8a** was >90% in all solvents studied. The rate constants for **5a** in variable-temperature studies in acetonitrile (Supporting Information) gave an Arrhenius function of $\log k = (11.7 \pm 0.2) - (7.6 \pm 0.2)/2.3RT$ in kcal/mol with errors at 2σ, and the entropic term is consistent with those found in other 3-*exo* radical cyclizations.[10,11] Compound **9a** was the only product observed in the NMR spectrum of the crude mixture from reaction of **4a** with Bu_3SnH in acetonitrile-d_3.

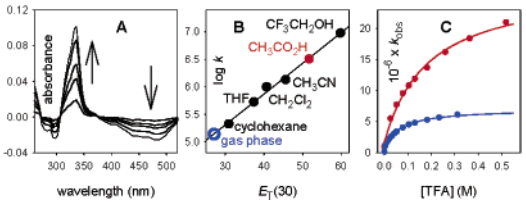
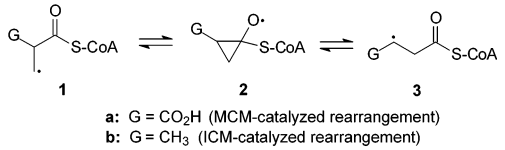
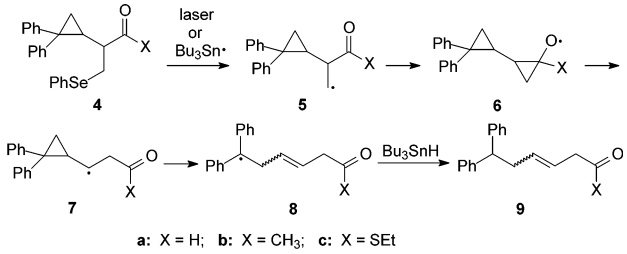


Figure 1. (A) Time-resolved spectrum from reaction of **5a** in CH_3CN. The traces are at 0.3, 0.5, 0.7, 1.3, and 2.5 μs with signals at 0.14 μs subtracted to give a baseline; product **8a** is growing in, and the phenylselenyl radical is decaying. (B) Observed rate constants (solid circles) for reactions of radical **5a** at 20 °C. (C) Rate constants for reactions of **5a** in the presence of CF_3CO_2H in CH_2Cl_2 (red) and in cyclohexane (blue); the lines are fits described in Supporting Information.

Scheme 1



Scheme 2



The rate constants for rearrangement of radical **5a** varied as a function of solvent polarity as shown in Figure 1B, where rate constants at 20 °C are plotted against $E_T(30)$ solvent polarity values.[12] The solvent effect results from polarization in the transition state for the radical addition reaction (**5a** → **6a**), consistent with the nucleophilic character of alkyl radicals.[13]

The methyl ketone radical **5b** reacted less rapidly than aldehyde **5a**; the rate constant for rearrangement in 2,2,2-trifluoroethanol (TFE) at 20 °C was $k = 1 \times 10^5$ s^{-1}. The steric effect of the methyl group in **5b**, a decrease in rate by 2 orders of magnitude, is similar to those in other 3-*exo* radical cyclizations.[11] In LFP studies of radical **5c**, we observed no growth of signal from rearrangement product **8c**, even in TFE, which establishes a limit for the rate

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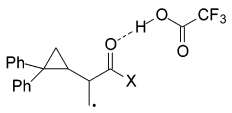
constant of $k < 1 \times 10^4$ s^{-1}; a small rate constant for **5c** was expected from experimental[4] and computational[5] results.

From Figure 1B, it is apparent that acetic acid imparted a normal solvent polarity effect on the kinetics but no special acid catalysis. Minor acid catalysis of the rearrangements of radicals **5a** and **5b** could be observed with the strong acid CF_3CO_2H, however, as shown for **5a** in Figure 1C. The increase in rate is due to reaction of the hydrogen-bonded complex **10a**. The data were solved for reversible complexation followed by rate-limiting rearrangement (Supporting Information). The rate constants for reaction of acid-complexed radical **10a** in cyclohexane and in CH_2Cl_2 at 20 °C are $k = 7 \times 10^6$ s^{-1} and $k = 3 \times 10^7$ s^{-1}, respectively. For reaction of the methyl ketone radical in CH_2Cl_2 with CF_3CO_2H, the rate constants were $k = 4 \times 10^5$ s^{-1} for complex **10b** and $k = 0.6 \times 10^5$ s^{-1} for neutral radical **5b**.



10a: X = H; **10b:** X = CH_3

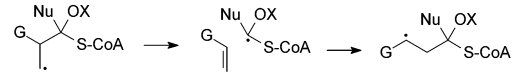
The agreement between experimentally determined rate constants and computed reaction barriers is good for the neutral radicals. Extrapolation of the rate constants for **5a** to $E_T(30) = 27.1$, the value for the gas phase,[12] gives a rate constant of $k = 1.4 \times 10^5$ s^{-1} at 20 °C (Figure 1B). Using this rate constant and log $A = 11.7$ gives $E_a = 8.8$ kcal/mol. For comparison, the computed barrier for cyclization of the propanal-3-yl radical in the gas phase is $\Delta E = 9.5-12.4$ kcal/mol.[5]

For "partially protonated" radicals, the agreement between experiment and theory is poor. Complexation with the strong acid CF_3CO_2H resulted in an order of magnitude rate acceleration, and no catalysis was apparent in neat acetic acid. Smith et al. computed that the barriers for cyclizations of models of the methylmalonyl-CoA radical would be reduced by 50−80% upon protonation or complexation with H_3O^+ and reasoned that carboxylic acid-complexed radicals would react as fast as the H_3O^+ complexes.[5]

Our results show moderate solvent effects for a radical addition to a carbonyl group and indicate that a weak acid in an enzyme will not provide significant catalysis for the associative radical reaction in Scheme 1. This conclusion presents a conundrum for the rearrangements catalyzed by MCM and ICM enzymes where the weak acid histidine is a potential catalyst.[14,15] The MCM enzyme turns over succinyl-CoA with $k_{cat} = 48$ s^{-1},[16] but the computed barrier for cyclization of the neutral succinyl-CoA radical is 23−26 kcal/mol[5] ($k \approx 1 \times 10^{-7}$ s^{-1}). Thus, the associative pathway is kinetically incompetent for the enzyme-catalyzed process by many orders of magnitude. Nonetheless, His244 in MCM apparently has a catalytic role. It is adjacent to substrates in crystal structures with imidazole nitrogen close to the thioester groups,[17] and MCM mutants H244G, H244A, and H244Q display activity that is attenuated by 2−3 orders of magnitude.[14,15]

A radical center offers two distinct reactivity patterns, an associative pathway as in Scheme 1 and a dissociative pathway

Scheme 3



11: X = ⊖
12: X = H

that exploits the reduction in bond energies to groups vicinal to the radical center. Homolysis reactions of model radicals for the MCM-catalyzed reaction, giving an acyl radical and an alkene, were computed to be relatively high-energy processes;[5,6] thus, a dissociative pathway that was presented in a unified mechanistic proposal for coenzyme B_{12}-dependent mutase enzymes[18] was disfavored. With the finding that the associative pathway is not strongly acid catalyzed, we suggest that the dissociative route be revisited in a modified form. It seems possible that the bond energies of distonic radical anions **11** or adducts **12** could be small. Perhaps His244 in MCM functions as a nucleophilic catalyst or as a catalyst for addition of water to the thioester group of substrate to give adduct radicals that react in a dissociative process such as shown in Scheme 3. We suggest that these complex pathways should be evaluated.

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Supporting Information Available: Kinetic results, synthetic details, and NMR spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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