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Kinetic Results Implicating a Polar Radical Reaction Pathway in the Rearrangement Catalyzed by α-Methyleneglutarate Mutase

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Abstract: α-Methyleneglutarate mutase (MGM) catalyzes the rearrangement of 2-methyleneglutarate to 3-methylitaconate (2-methylene-3-methylsuccinate). A putative mechanism for the MGM-catalyzed reaction involves 3-*exo* cyclization of the 2-methyleneglutaric acid-4-yl radical to a cyclopropylcarbinyl radical intermediate that ring opens to the 3-hydroxycarbonyl-2-methylenebutanoic acid-4-yl radical (3-methylitaconic acid radical). Model reactions for this mechanism were studied by laser flash photolysis kinetic methods. α-Ester radicals were produced by 266 nm photolysis of α-phenylselenyl ester derivatives. Rate constants for cyclizations of the (*Z*)-1-ethoxycarbonyl-4-(2,2-diphenylcyclopropyl)-3-buten-1-yl radical ((*Z*)-**8a**) and (*E*)- and (*Z*)-1,3-di(ethoxycarbonyl)-4-(2,2-diphenylcyclopropyl)-3-buten-1-yl radicals ((*E*)- and (*Z*)-**8b**) were determined. The ester group in (*Z*)-**8a** accelerates the 3-*exo* cyclization in comparison to the parent radical lacking an ester group by a factor of 3, an effect ascribed to a polarized transition state. The ester groups at C3 in radicals **8b** slow the 3-*exo* cyclization reaction by a factor of 50. The rate constant for cyclization of the 2-methyleneglutaric acid-4-yl radical is estimated to be $k \approx 2000\ s^{-1}$ at ambient temperature. When coupled with the estimated partitioning of the intermediate cyclopropylcarbinyl radical, the overall rate constant for the conversion is estimated to be $k \approx 1 \times 10^{-3}\ s^{-1}$, which is much too small for any radical reaction and several orders of magnitude too small for kinetic competence for the MGM-catalyzed process. The possibility that the radical reaction in nature involves an unusual mechanism in which polar effects are important is discussed.

Nature effects a variety of molecular rearrangement reactions via radical intermediates.[1−4] The reactions typically involve breaking and forming high-energy carbon−carbon or carbon−heteroatom bonds, and the radical manifold provides pathways inherently lower in energy than those available for closed-shell species. One family of biological rearrangements is catalyzed by adenosylcobalamin (coenzyme B_{12})-dependent enzymes that initiate nonchain radical reaction sequences by homolysis of the cobalt−carbon bond of the coenzyme, producing the 5′-deoxyadenosin-5′-yl radical.[1,2,5−7] The deoxyadenosinyl radical abstracts a hydrogen atom from the substrate, and a radical rearrangement involving a one-atom migration of a group ensues. 5′-Deoxyadenosine subsequently donates a hydrogen atom to the product radical, and the 5′-deoxyadenosin-5′-yl radical recombines with cobalt to regenerate coenzyme B_{12} and complete the reaction cycle. Examples include interconversions of methylmalonyl-CoA with succinyl-CoA,[1] glutamate with 3-methylaspartate,[3,5,7] and 2-methyleneglutarate with 3-methylitaconate.[8,9]

The mechanisms by which the radical rearrangements occur are subjects of interest because the reactions have limited analogies in organic radical chemistry. One of the seemingly least controversial rearrangements is that catalyzed by α-methyleneglutarate mutase (MGM), which isomerizes 2-methyleneglutarate to 3-methylitaconate (Scheme 1).[8,9] It would appear that the pathway shown in the scheme is reasonable, with reversible conversion of the 2-methyleneglutarate radical (**1a**) to the 3-methylitaconate radical (**3a**) via the intermediate cyclopropylcarbinyl radical **2a**. Ashwell et al.[10] found that both diastereomers of radical **2b** opened to radical **1b** and that radical **3b** partially isomerized to radical **1b** in competition with trapping by a tin hydride reagent. Those results support the possibility of the pathway shown in Scheme 1, although radical **1b** gave no rearrangement products when it was generated.[10]

Nonetheless, recent results indicate that the mechanism in Scheme 1 is not the pathway for the MGM-catalyzed rearrangement. In a high-level computational study, Smith et al.[9] found that the overall conversion of **1a** to **3a** should have a rate constant of $k < 0.01\ s^{-1}$.[11] As discussed later, such a rate

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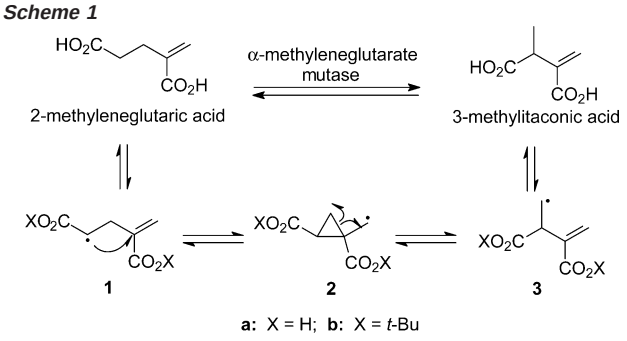
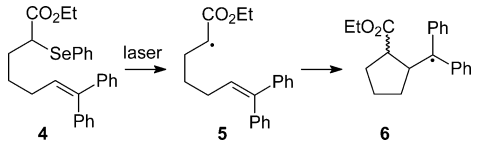
Scheme 1



Scheme 2



constant is too small for an efficient radical reaction, and it is orders of magnitude too small for kinetic competence in the enzyme-catalyzed rearrangement.[8,12] Moreover, isotope-labeling studies of the enzyme-catalyzed reaction indicate that a fragmentation−recombination reaction pathway is likely.[8] Thus, the mechanism in Scheme 1 has no computational or experimental support except evidence that the strongly thermodynamically favored "back reaction" of **3b** to **1b** is possible.[10] We report kinetic studies of models for the "forward reaction", the cyclization of radical **1**. Our results support the conclusions that the mechanism in Scheme 1 is not likely. We propose that the rearrangement in nature is likely to involve an unusual polar radical reaction mechanism.

Results

In this work, we produced α-ester radicals by laser photolysis of α-phenylselenyl ester derivatives. Products from photolyses of alkyl phenyl selenides have been studied,[13] but applications of such radical precursors in laser flash photolysis (LFP) studies have not been reported previously. Details of the study of radical precursor **4** (Scheme 2) will be forthcoming,[14] but for the purpose of the present work we used this system to provide a method for quantitating yields of diphenylalkyl radicals.

Photolysis of **4** gave the known[15] α-ester radical **5** that cyclized to radical **6** (Scheme 2). The spectrum of radical **6** is shown in Figure 1. The observed spectrum is a combination of the spectrum from **6** and the spectrum from the phenylselenyl radical. At wavelengths shorter than 300 nm, the spectrum consists of an intense signal from the phenylselenyl radical ($\lambda_{max} \approx 285$ nm), and end absorption from this signal extends to about 330 nm. The phenylselenyl radical spectrum also is shown in Figure 1 (green trace), and subtraction of the

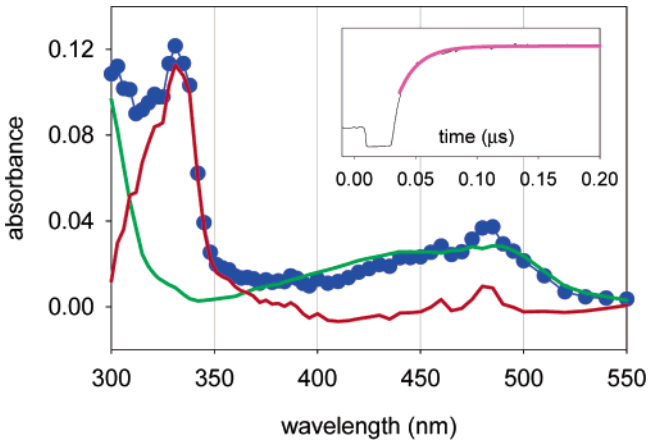


Figure 1. Spectrum of radical **6** in acetonitrile. The blue line is the spectrum observed 125 ns after the laser pulse where the symbols show the monitored wavelengths. The green line is the spectrum of the phenylselenyl radical (from photolysis of diphenyl diselenide) scaled to minimize the absorbance differences from the observed data in the long wavelength range. The red line is the difference spectrum due to radical **6**. The inset shows the kinetic trace at 335 nm (black line) fit to a first-order exponential growth (pink line); PM tube recovery from the laser flash and fluoresence occurs in the first 30 ns.

phenylselenyl radical spectrum from the observed spectrum gave the spectrum of **6**, which is the red trace in Figure 1. The noteworthy features of this spectrum are that the long wavelength λ_{max} is at ca. 335 nm and the absorbance drops off rapidly at longer wavelengths. Many similar spectra of diphenylalkyl radicals with λ_{max} in the 332−338 nm range and the "signature" rapid drop-off in absorbance at longer wavelengths have been reported.[15−19]

The cyclization reaction of radical **5** is sufficiently fast ($k = 4 \times 10^7$ s^{-1} at ambient temperature)[15] that other reactions do not compete. Therefore, the maximum intensity of the signal at λ_{max} for **6** relative to a signal due solely to the phenylselenyl radical can be used for calibrating yield determinations of other diphenylalkyl radicals. In acetonitrile, the signal at ca. 335 nm from **6** radical grew to a maximum value of 0.11 AU when the instantaneous signal intensity at 490 nm due to the phenylselenyl radical was 0.03 AU. In studies discussed below, we use the ratios of the signals at λ_{max} for diphenylalkyl radicals relative to the signal intensity at 490 nm from phenylselenyl to determine the yields of diphenylalkyl radicals, where a ratio of 3.7 is taken to be 100% yield. Diphenylalkyl radicals have ϵ values of ca. 20 000 AU cm^{-1} M^{-1},[20] and the concentration of radical **6** in the spectrum in Figure 1 was approximately 6×10^{-6} M.

To study the 3-*exo* radical cyclization of **1**, the intermediate cyclopropylcarbinyl radical **2** or the thermodynamically unfavored radical **3** must be trapped efficiently. Nature can overcome an unfavorable equilibrium by selectively trapping radical **3a**. In model radical studies, we incorporated an intramolecular trapping reaction to divert the cyclopropylcarbinyl radical

(11) Smith et al.[9] used basis sets that give excellent results for the barrier for cyclization of the 3-butenyl radical. The high-energy point for the conversion of **1a** to **3a** via the pathway in Scheme 1 was 20−21 kcal/mol above the energy of radical **1a**. Because 3-*exo* radical cyclizations have a low entropy demand, the computed barrier is nearly equal to the free energy of activation of the reaction.
(12) Recently determined value for the enzyme-catalyzed conversion of 2-methyleneglutarate is $k_{cat} = 30$ s^{-1}; see ref 8.
(13) Kropp, P. J.; Fryxell, G. E.; Tubergen, M. W.; Hager, M. W.; Harris, G. D.; McDermott, T. P.; Tornero-Velez, R. *J. Am. Chem. Soc.* **1991**, *113*, 7300−7310.
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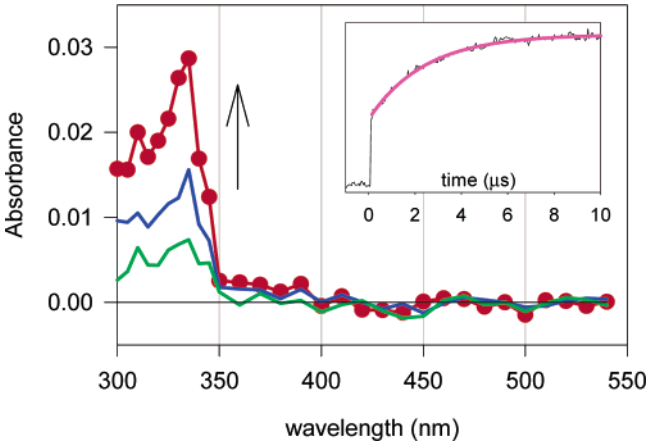
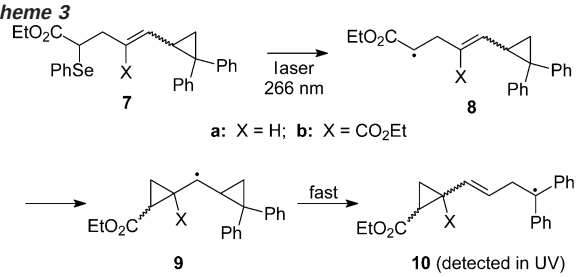


Figure 2. Time-resolved growth spectrum for radical **10a** in acetonitrile. The traces show absorbance at 1 μs (green), 2 μs (blue), and 6 μs (red) after the laser pulse with the absorbance at 0.3 μs subtracted to give a baseline. Symbols on the 6 μs trace show the monitored wavelengths. The inset shows a kinetic trace at 335 nm (black line) fit with a first-order exponential growth function (pink line).

Scheme 3



formed by 3-*exo* cyclization.[21] The experimental design is shown in Scheme 3. α-Phenylselenyl ester radical precursors **7** were cleaved photochemically to give radicals **8** that could cyclize to radicals **9**. The diphenylcyclopropylcarbinyl radical moiety in radicals **9** provides the intramolecular trap. Ring opening of **9** to give the diphenylalkyl radical **10** is expected to be more than 1 order of magnitude faster than ring opening of an ester-substituted cyclopropylcarbinyl radical.[22] Thus, the fast ring opening of the 2,2-diphenylcyclopropylcarbinyl moiety effectively diverts **9** to diphenylalkyl radical **10**, which can be detected readily due to the strong diphenylalkyl radical chromophore.

Laser photolysis of solutions containing radical precursor (*Z*)-**7a** with 266 nm light gave, in addition to the spectrum of the phenylselenyl radical, another spectrum with $\lambda_{max} = 338$ nm that grew in with time. Figure 2 shows a result where we subtracted the spectral data at a short time so that the instantaneous signal from the phenylselenyl radical was removed; thus, the spectra show only the peaks changing in intensity between 0.3 and 6 μs. The growing spectrum is quite similar to that from radical **6** and other diphenylalkyl radicals, and we assign the spectrum to radical **10a**. The total intensity growth in Figure 2 is less than that in Figure 1 because the radical concentrations are smaller, ca. 2×10^{-6} M. In acetonitrile, the ratio of the final absorbance at λ_{max} relative to the instantaneous absorbance at 490 nm was 3.3, indicating that the yield of product radical **10a** was about 90%.

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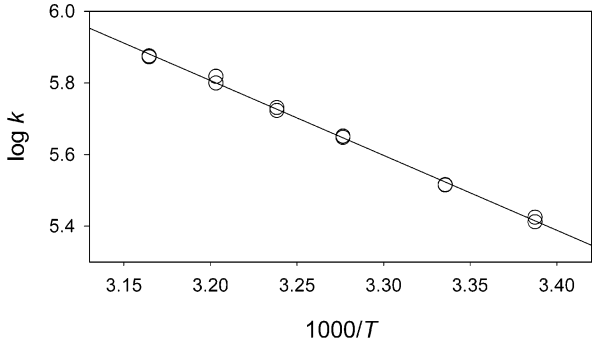


Figure 3. Temperature-dependent function for reaction of radical **8a** in acetonitrile. Two kinetic measurements are shown at each temperature. The line is eq 1.

Kinetic studies of radical (*Z*)-**8a** in various solvents at 22 °C gave the following rate constants: 2.4×10^5 s^{-1} (THF); 4.9×10^5 s^{-1} (acetonitrile); 1.3×10^6 s^{-1} (2,2,2-trifluoroethanol (TFE)−acetonitrile, 20:80, v:v); 1.1×10^6 s^{-1} (water−acetonitrile, 50:50, v:v). An increase in the rate constant for cyclization of radical (*Z*)-**8a** with increasing solvent polarity was apparent. In an attempt to observe acid catalysis of the reaction, kinetic studies were performed with acetonitrile solutions containing 0.005 and 0.01 M trifluoroacetic acid, but no change in rate constant was found; the precursor was destroyed in the presence of more concentrated acid. Variable temperature studies in acetonitrile (data in Supporting Information) gave the Arrhenius function in eq 1 (Figure 3), where errors are at 2σ and $\theta = 2.3RT$ in kcal/mol.

$$\log k_{obs} = (12.5 \pm 0.2) - (9.5 \pm 0.3)/\theta \qquad (1)$$

The measured rate constants in the LFP studies are the sums of the rate constants for all reactions that deplete (*Z*)-**8a**, and radical−radical termination reactions will make a contribution to the total rate. The observed pseudo-first-order rate constant for decay of the phenylselenyl radical signal via radical−radical reactions at 22 °C was ca. 1×10^5 s^{-1}. If decay of radical (*Z*)-**8a** via radical−radical reactions occurred with the same rate constant, then the observed rate constant for reaction of (*Z*)-**8a** in acetonitrile would be 20% greater than the rate constant for cyclization, and the yield of radical **10a** would be 80%. The agreement between the yield determined from the spectroscopic results (90%) and that estimated from the kinetic results is good. In 80:20 acetonitrile/TFE, the observed rate constant for reaction of (*Z*)-**8a** is estimated to be 5−10% greater than the rate constant for cyclization.

The ester groups at positions C3 of butenyl radicals **8b** were expected to impose substantial barriers to the 3-*exo* cyclizations. When LFP studies were conducted with radical precursors (*E*)-**7b** and (*Z*)-**7b**, the phenylselenyl radical spectrum was observed instantly, but no subsequent growth of signal was observed in any portion of the spectrum. Reactions were conducted in THF, in acetonitrile, in aqueous acetonitrile (50:50, v:v), and in TFE−acetonitrile (20:80, v:v) with low initial radical concentrations ($2-3 \times 10^{-6}$ M) to slow bimolecular radical−radical termination processes. In all of the experiments, decay was observed in the range 335−338 nm, the expected λ_{max} of radicals **10b**, following the initial absorbance created by the laser pulse. Thus, the intensity of any signal growth from **10b** was less than the intensity of signal decay from the phenylselenyl radical. The

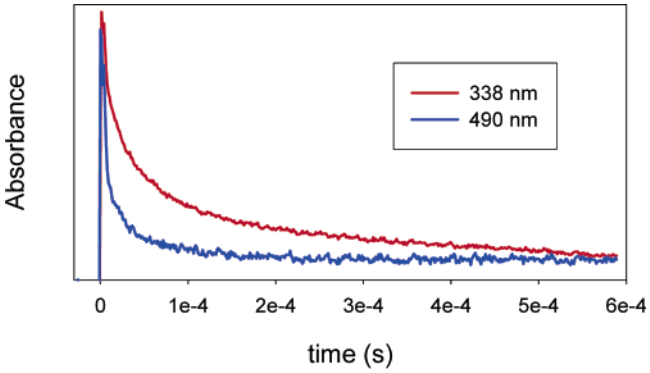


Figure 4. Kinetic traces at 338 and 490 nm from photolysis of (*Z*)-**7b**. Data have been scaled for comparison, and absorbance is in arbitrary units.

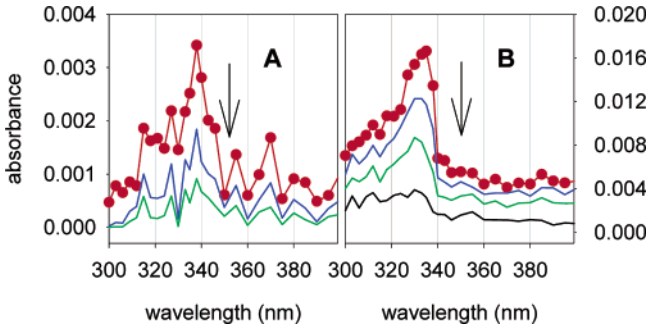


Figure 5. Time-resolved decay spectra for radicals **10b** at 22 °C. (A) Spectra from (*E*)-**8b** at 50 μs (red), 150 μs (blue), and 250 μs (green) with signals at 450 μs subtracted to give a baseline. (B) Spectra from (*Z*)-**8b** at 45 μs (red), 77 μs (blue), 141 μs (green), and 333 μs (black) with signals at 589 μs subtracted to give a baseline. Symbols on the red traces show monitored wavelengths.

observed pseudo-first-order rate constants for phenylselenyl radical decay determined at 490 nm were ca. 1×10^5 s^{-1}. From the relative molar extinction coefficients of diphenylalkyl radicals and the phenylselenyl radical at 335−338 nm, the results require that the rate constants for formation of radicals **10b** at 22 °C were $k < 5 \times 10^4$ s^{-1}.

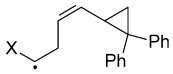
Figure 4 shows the decay behavior of kinetic traces from an experiment with (*Z*)-**7b**. Signal decay in Figure 4 is complex, but the 490 nm signal clearly decays much faster than the 338 nm signal. The phenylselenyl radical, which is the only species absorbing at 490 nm, was essentially depleted within 100 μs. The slow decay portion of the 338 nm trace nm has a pseudo-first-order rate constant of 6×10^3 s^{-1}, and the absorbance must be due to other radicals, which in this experiment can only be product radicals **10b**. Diphenylalkyl radicals decay relatively slowly due to radical stability and steric effects.[17,19−21,23,24]

The production of radicals **10b** was confirmed by monitoring the signal decay *after* 50 μs. Figure 5 shows typical results. These are time-resolved decay spectra where the signals at long times (ca. 500 μs) were subtracted from signals at shorter times. Figure 5A shows results from an experiment with (*E*)-**7b** where the initial concentration of each radical was ca. 2×10^{-6} M, and Figure 5B shows results from an experiment with (*Z*)-**7b** where the initial concentration of each radical was ca. 3×10^{-6} M. The improved signal-to-noise in the spectrum in Figure 5B resulted from the use of oversampling methods (64:1) to reduce noise. The decay spectra clearly resemble the spectrum of radical **10a** (cf. Figure 2), as expected.

The yields of radicals **10b** observed in the decay spectra were determined from the ratios of absorbances at λ_{max} at ca. 50 μs and the instantaneous signals at 490 nm from phenylselenyl. In several experiments conducted in TFE−acetonitrile (20:80, v:v), the yields of **10b** were in the range 10−18%, and we estimate that the maximum yield of radicals **10b** was about 20%. The cyclization reactions of radicals **8b** compete with radical−radical coupling reactions. If we make the reasonable assumption that the total pseudo-first-order rate constants for reactions of α-ester radicals **8b** were similar to that of the phenylselenyl radical ($k_{decay} \approx 1 \times 10^5$ s^{-1}), then the rate constants for cyclization reactions of radicals **8b** at 22 °C are $k \approx 2 \times 10^4$ s^{-1}.

Discussion

Rate Constants for 3-*exo* Radical Cyclizations. The effects of the two carboxylic acid (or ester) groups in the cyclization of the α-methyleneglutarate radical can be evaluated independently. Although seemingly counterintuitive, the terminal ester group in radical (*Z*)-**8a** *accelerates* the cyclization reaction. The rate constant for cyclization of (*Z*)-**8a** in THF ($k \approx 2 \times 10^5$ s^{-1}) is a factor of 3 greater than the rate constant for cyclization of radical **11** in the same solvent.[21] This acceleration undoubtedly results from a polarization of the transition state for cyclization of (*Z*)-**8a** due to the ester group. Similar kinetic effects of α-ester groups have been found in 5-*exo* radical cyclizations[15] and in cyclopropylcarbinyl radical ring openings.[24] The observed increase in the rate constant for cyclization of radical (*Z*)-**8a** as the solvent polarity was increased is another indication of a polarized transition state; such solvent polarity effects are not found in simple alkyl radical reactions.



(*Z*)-**8a**: X = CO_2Et
11: X = H

A polarized transition state for the 3-*exo* radical cyclization reaction of radical **1a** (Scheme 1) should result in a small overestimation of the barrier for cyclization of **1a** in the computational study of the reaction because neither a dielectric continuum nor specific solvent molecule effects were incorporated in the calculations.[9] However, a reduction in the computed barrier for this reaction will not have an effect on the overall barrier for conversion of the 2-methyleneglutaric acid radical **1a** to the 3-methylitaconic acid radical **3a**. Polarization is not expected in the transition state for the ring opening of radical **2a** that gives radical **3a**, and this process passes through the high-energy point of the overall reaction.[9]

The ester groups at C3 in butenyl radicals **8b** have dramatic steric effects on the cyclization reactions. The approximate rate constant for cyclization of these radicals in TFE−acetonitrile (20:80) at 22 °C is $k \approx 2 \times 10^4$ s^{-1}, which represents a reduction of the cyclization rate constant in comparison to that for radical (*Z*)-**8a** in the same solvent mixture by a factor of 50. The steric effect is similar to that found in 5-*exo* radical cyclizations where the methyl group at C5 in radical **12b** reduces the rate constant

(23) Halgren, T. A.; Roberts, J. D.; Horner, J. H.; Martinez, F. N.; Tronche, C.; Newcomb, M. *J. Am. Chem. Soc.* **2000**, *122*, 2988−2994.
(24) Horner, J. H.; Tanaka, N.; Newcomb, M. *J. Am. Chem. Soc.* **1998**, *120*, 10379−10390.

by a factor of approximately 50 at 65 °C compared to the rate constant for cyclization of radical **12a**.[25]

12a: R = H
12b: R = CH_3

Rate Constants and Energetics of the Pathway in Scheme 1. For comparison of the results with model radicals **8b** to the 2-methyleneglutarate radical, an accelerating effect of the diphenylcyclopropane reporter group in radicals **8b** should be factored out of the kinetics. In the case of 3-*exo* cyclizations of 3-butenyl radicals, the same reporter group as that used here accelerates the cyclizations by a factor of 7 and 10 for the (*E*)- and (*Z*)-isomers, respectively.[21] Therefore, we estimate the rate constant for the 3-*exo* cyclization of the diacid or diester radical lacking the reporter group (i.e., the diethyl ester analogue of radical **1b** in Scheme 1) to be $k \approx 2 \times 10^3$ s^{-1} at ambient temperature. The rate constant for cyclization of radical **1a** should be similar in magnitude.

The deduced rate constant for cyclization of radical **1a** might appear to be acceptable for the MGM-catalyzed radical conversion, but it is not. Following the cyclization of radical **1a**, the intermediate cyclopropylcarbinyl radical **2a** will partition preferentially back to radical **1a**. An estimated partition factor using kinetic results for ring openings of the cyclopropylcarbinyl radical[23] and the 2-(ethoxycarbonyl)cyclopropylcarbinyl radical[26] is 2000:1, favoring **1a** over **3a**, but the use of these models is suspect because both lack the substituent at C1 of the cyclopropyl ring. From the computational results of Smith et al.,[9] the partitioning of **2a** would favor radical **1a** by a factor of 3×10^6 if the two ring-opening processes have the same entropy demand. Using the computed partition factor and the rate constant for the cyclization reaction, the overall rate constant for conversion of **1a** to **3a** would be $k \approx 1 \times 10^{-3}$ s^{-1}.

Even a generous estimate of the rate constant for rearrangement of **1a** to **3a** of ca. 1 s^{-1} leads to the conclusion that the simple 3-*exo* radical cyclization pathway in Scheme 1 is unlikely to be the reaction pathway in nature. The reaction would be a factor of 30 smaller than the observed k_{cat} in the MGM-catalyzed reaction,[8,12] but that is not the major problem with such a slow radical reaction because it is unlikely that the rate-limiting reaction in the enzyme-catalyzed process is the radical rearrangement. Specifically, a carbon-centered radical in an enzyme would have to be protected from reactions with the low bond energy α-hydrogen atoms of peptides to achieve a long (0.03 s) lifetime. In condensed phase, reactions of carbon-centered radicals that have rate constants smaller than 100 s^{-1} at ambient temperature are not efficient; these radicals react with bulk solvents such as THF or benzene with pseudo-first-order rate constants on the order of 100−1000 s^{-1}.

A major purpose of our study was to evaluate the computational results for the MGM-catalyzed reaction.[9] If those results are correct, the rearrangement of **1a** to **3a** via the pathway in Scheme 1 is about 6 orders of magnitude too slow to be reasonable for a radical reaction and nearly 5 orders of magnitude too slow for kinetic competence in regard to k_{cat}. Our experimental results and those of Ashwell et al.[10] can be used to evaluate the computed barriers. Figure 6 shows the computed barrier heights for 3-*exo* cyclizations of radicals **1a** and **3a** and the computed difference in ground-state energies for these two radicals.[9] Also shown in Figure 6 are the enthalpies of activation for the cyclization reactions of radicals **1a** and **3a**.

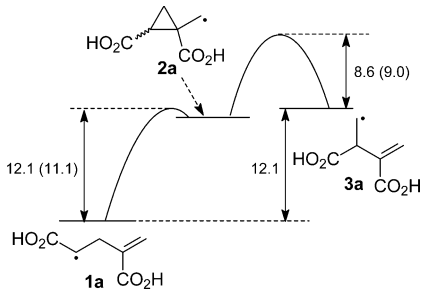


Figure 6. Relative energies of radicals **1a** and **3a** and barriers for their 3-*exo* cyclization reactions in kcal/mol. The computed energy values determined in ref 9 are given, and experimental enthalpies of activation (see text) are in parentheses.

The experimental enthalpies of activation were obtained in the following manner. The rate constant for cyclization of radical **1a** was taken to be 2000 s^{-1} at 22 °C from this work. The rate constant for cyclization of radical **3a** was obtained from the results of Ashwell et al.,[10] who found a 2:1 ratio of rearranged to unrearranged products when radical **3b** was produced at 50 °C in the presence of 0.01 M Ph_3SnH. Using the accepted rate constant for reaction of Ph_3SnH with an alkyl radical,[27] one calculates a rate constant for rearrangement of **3b** at 50 °C of $k = 3 \times 10^5$ s^{-1}. The log *A* values, the entropy terms, for the 3-*exo* cyclizations were taken to be 12, which is an average log *A* value from several experimental determinations of Arrhenius functions for 3-*exo* radical cyclizations, including that determined in this work.[21,23] From these values, one calculates activation energies for the cyclizations and enthalpies of activation, where $\Delta H^{\ddagger} = E_a - RT$. For radical **3**, $\Delta H^{\ddagger} = 9.0$ kcal/mol, and $\Delta H^{\ddagger} = 11.1$ kcal/mol for radical **1**. The agreement between the computed barriers and the activation enthalpies is quite good. As noted earlier, the computed barrier for cyclization of **1a** should be somewhat high because the computations do not account for solvent effects on the polarized transition state for this cyclization.

The agreement between the computed barriers and the activation energies indicates that the computational methods handled the difficult calculations of the transition state energies quite well. If the difference in energies between radicals **1a** and **3a** was computed with the same level of accuracy, which is quite likely, then the total barrier for conversion of **1a** to **3a** found computationally, 20.7 kcal/mol, is expected to be accurate. Thus, experimental results support the computational conclusion that conversion of **1a** to **3a** is nearly 5 orders of magnitude too slow for kinetic competence in the enzyme-catalyzed isomerization reaction. We note that the ground-state energy of the intermediate cyclopropylcarbinyl radical is inconsequential.

Mechanism of the MGM-Catalyzed Rearrangement. One rationalization for the failure of the model radical reactions and

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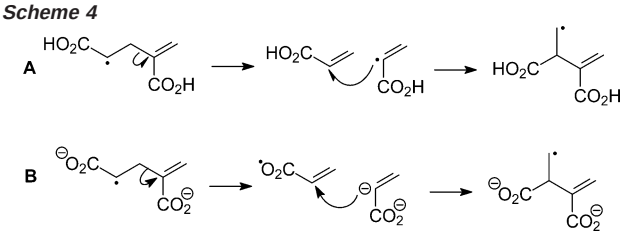
the computations to confirm the viability of the pathway in Scheme 1 is that the enzyme alters the radical rate constants by steric effects, but that premise seems unlikely for this case. The transition states for 3-*exo* radical cyclizations already have essentially no entropy penalty,[23] as shown in the log *A* term in eq 1, and there is only one free rotor in radical **1a** that the enzyme could restrict. Possible favorable binding of the cyclic radical is not an issue, because that would only change the energy level of the intermediate, which is of little consequence for the total barrier height. In the MGM studies, Pierik et al.[8] found that K_M for the reactant was 8 times larger than K_M for the product, and a similar difference in binding energies of radicals **1a** and **3a** might be expected. If that translates into a reduced energy for the barrier to the product radical, then the reaction of radical **1a** remains about 4 orders of magnitude too slow for kinetic competence.

In the context of reactions of carboxylic acid-substituted radicals, protonation of the double bond would reduce the barrier for the cyclization. Smith et al.[9] noted in passing that "partial protonation" of the alkene group would reduce the energy manifold in the MGM-catalyzed rearrangement, but it is difficult to envision partial protonation of an alkene by any acid available in the enzyme. Our studies of the cyclization reaction of radical **8a** in the presence of the strong acid CF_3CO_2H were aimed at uncovering an acid-catalyzed rate enhancement, but we found no kinetic effect.

The obvious alternative for the failure of the model reactions and computations to confirm the pathway in Scheme 1 is that the pathway is not correct. At this time, one has experimental kinetic data for the forward reaction from this work, kinetic data for the back reaction,[10] and high-level computational results[9] that indicate that the pathway is not energetically accessible. In addition, labeling studies[8] indicated that the most likely pathway involves a dissociation−recombination sequence, not an associative pathway such as that in Scheme 1. On the other hand, no experimental evidence supports the viability of the pathway in Scheme 1. We conclude that the model reaction is fundamentally flawed, either in the nature of the radicals, in the mechanism of the reaction, or in both.

The issue of the nature of the radicals is, we believe, quite important. The computations were performed for neutral radicals,[9] not for charged α- and *β*-carboxylate radicals, which would have been challenging to address. In nature, however, the radicals most likely maintain a negative charge on the carboxylate groups, and the "radicals" are actually radical anions. It is possible that the energy difference between α- and *β*-carboxylate-substituted radicals is smaller than that between α- and *β*-carboxylic acid and ester radicals. This is indicated in the computational results of Dybala-Defratyka and Paneth,[28] who studied the isomerization of methylmalonyl-CoA to succinyl-CoA. They[28] found that the energy difference between the ground states of α- and *β*-carboxylate radicals was about 3 kcal/mol smaller than that between the corresponding α- and *β*-carboxylic acid radicals. Such a change in the energy gap between radicals **1a** and **3a** might make the cyclization−fragmentation pathway of Scheme 1 energetically feasible. Of course, the entire energy profile would need to be addressed for radical anions, and this will undoubtedly be difficult because polar effects in the enzymes are likely to be important for radical anion reactions.

Scheme 4



Just as the computations addressed what might be the "wrong" radicals, so did the kinetic studies. We and Ashwell et al.[10] studied neutral, ester-substituted radicals. The kinetic studies test the computational results, but they do not necessarily test the reaction pathway in nature. To do that, one might need to develop new methods for production of α- and *β*-carboxylate radicals. Simple α-carboxylate radicals such as that from acetate have been produced by X-irradiation of glycine,[29,30] by abstraction of hydrogen from acetate in pulse radiolysis reactions,[31] and in the gas phase in ion cyclotron resonance studies,[32] but these methods are not amenable to sophisticated models. We are not aware of studies of *β*-carboxylate-substituted radicals.

New computational and experimental methods might be developed for studies of the associative pathway of Scheme 1 with carboxylate-substituted radicals, but the labeling studies of Pierik et al.[8] suggest that the pathway is dissociative; that is, that the reaction occurs by a fragmentation−recombination sequence. The mechanism favored in that work was a homolytic dissociation to give a vinyl radical and acrylic acid, which then recombine by radical addition to the α-carbon of acrylic acid to give the *β*-substituted radical (Scheme 4, pathway A). This pathway seems doubtful because it is computed to have a 40 kcal/mol barrier.[9]

In our view, a more likely candidate mechanism for a dissociative pathway is heterolytic fragmentation of the carboxylate radical to give the vinylacyloxyl radical and a vinyl anion that recombines (pathway B in Scheme 4). Heterolytic fragmentation of a neutral radical such as **1a** would be a high-energy process, and this pathway is effectively excluded in the computations and kinetic studies by the selection of neutral models. However, heterolysis of a radical anion would not create charge but only redistribute it, and even neutral radicals heterolyze very rapidly (sub-nanosecond) when good leaving groups are involved and the solvent is modestly polar.[20]

In summary, kinetic studies support the computational predictions that the cyclization−ring-opening reaction pathway of neutral radicals is unlikely to be the mechanism of the α-methyleneglurate mutase-catalyzed rearrangement in nature. We believe that the reaction might involve carboxylate-substituted radicals, which offers the opportunity for polar stabilization effects in the enzyme that can be quite large, and we favor a heterolysis pathway such as B in Scheme 4 as a candidate mechanism that could be energetically accessible and would be in accordance with stereochemical studies of the MGM-catalyzed reaction. Carboxylate-substituted radicals, or more correctly radical anions, are essentially unknown both

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computationally and experimentally, but studies of these species would undoubtedly provide useful mechanistic information for the MGM-catalyzed rearrangement and other rearrangements catalyzed by coenzyme B_{12}-dependent enzymes.[3,4]

Experimental Section

Radical precursors were prepared from the corresponding esters by deprotonation with LDA followed by reaction with diphenyl diselenide. Synthetic details are contained in Supporting Information.

Laser flash photolysis studies were performed on LK-50 and LK-60 kinetic spectrometers (Applied Photophysics). Solutions of radical precursors in spectroscopic or HPLC-grade solvents (when available) were prepared such that the absorbances of the solutions at 266 nm were 0.4−0.5 AU. Solutions were sparged with helium and thermally equilibrated in a jacketed addition funnel and then allowed to flow through a 1 × 1 cm quartz cell. Temperatures were measured with a thermocouple placed in the flowing stream. Samples were irradiated with 266 nm light from a Nd-YAG laser (5 or 7 ns pulse). Data were acquired and analyzed with the Applied Photophysics software. Oversampling (64:1) was employed for weak signals in some cases. Kinetic results are listed in Supporting Information.

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

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