

## Carboxylate-Substituted Radicals from Phenylselenide Derivatives. Designs on Models for Coenzyme B<sub>12</sub>-Dependent Enzyme-Catalyzed Rearrangements

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Coenzyme B<sub>12</sub>-dependent enzymes catalyze a wide range of high-energy demanding rearrangement reactions wherein carbon–carbon or carbon–heteroatom bonds are broken and formed via radical intermediates.<sup>1,2</sup> A large subset of these reactions involves carboxylate substrates with interconversions between radicals that are  $\alpha$ - and  $\beta$ - to the carboxylate functionality. Examples in this group include equilibrations of methylmalonyl-CoA with succinyl-CoA<sup>3</sup> (Figure 1), glutamate with 3-methylaspartate,<sup>2,4</sup> and 2-methylene-glutarate with 2-methylene-3-methylsuccinate.<sup>2,5,6</sup> The mechanisms of these rearrangements are subjects of current research in part because few analogies are known in organic radical chemistry.

Studies of these intriguing reactions have been limited by the models available. Ester-substituted radicals have been studied experimentally,<sup>2,3,6,7</sup> and carboxylic acid-substituted radicals have been studied computationally,<sup>8</sup> but the reactive species in enzyme-catalyzed reactions are  $\alpha$ - and  $\beta$ -carboxylate radicals. Carboxylate-substituted radicals are actually radical anions, and they are likely to react differently than neutral radicals. Simple  $\alpha$ -carboxylate radicals, such as the radical anion from acetic acid, have been produced in condensed phase<sup>9</sup> and in the gas phase,<sup>10</sup> and more complex  $\alpha$ -carboxylate radicals have been inferred from products obtained by iodine oxidation of carboxylic acid dilithio derivatives.<sup>11</sup>  $\beta$ -Carboxylate radicals have not been described previously to our knowledge. In this work, we report that phenylselenenyl derivatives can be employed as precursors to specific  $\alpha$ - and  $\beta$ -carboxylate radicals in laser flash photolysis (LFP) kinetic studies conducted in aqueous solvent mixtures, and we demonstrate that neutral ester- and acid-substituted radicals are poor models for an  $\alpha$ -carboxylate radical.

General methods for synthesis of  $\alpha$ - and  $\beta$ -phenylselenenyl-substituted carboxylic acids were employed to prepare the radical precursors (Scheme 1). Thus, reactions of ester enolates with diphenyl diselenide and with phenylselenenylmethyl bromide gave  $\alpha$ - and  $\beta$ -phenylselenenyl esters, respectively, that were converted to the corresponding acids.

Production of  $\beta$ -ester,  $\beta$ -carboxylic acid, and  $\beta$ -carboxylate radicals was demonstrated with two systems that contain internal reporter groups<sup>12</sup> (Scheme 2). Photolyses of the  $\beta$ -phenylselenenyl derivatives **1** with 266 nm laser light gave radicals **2** that cyclized to observable radicals **3**. In a similar manner, the radical precursors **4** were cleaved by 266 nm light to give radicals **5**; 5-*exo* cyclizations of radicals **5** gave **6** containing the diphenylcyclopropyl reporter group, and fast ring openings of radicals **6** gave detectable radicals **7**. In all cases, time-resolved spectra showed fluorescence from the aryl groups that decayed within ca. 150 ns, the spectrum of the byproduct phenylselenenyl radical, and spectra of radicals **3** and **7**. Representative spectra are in the Supporting Information.

A consistent accelerating  $\beta$ -substituent effect for the carboxylic acid and carboxylate groups in radicals **2** and **5** was apparent in kinetic studies conducted in acetonitrile–water (1:1, v/v). At 22 °C, the 6-*exo* cyclizations had rate constants of  $5.0 \times 10^6 \text{ s}^{-1}$  (**2b**,

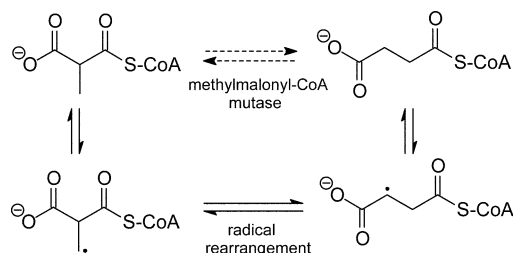
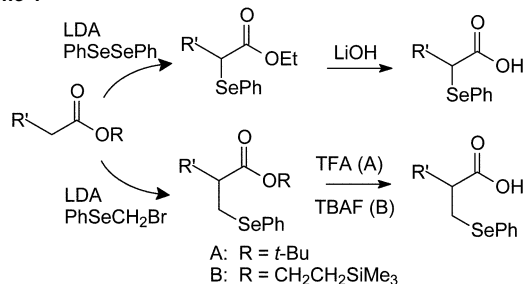
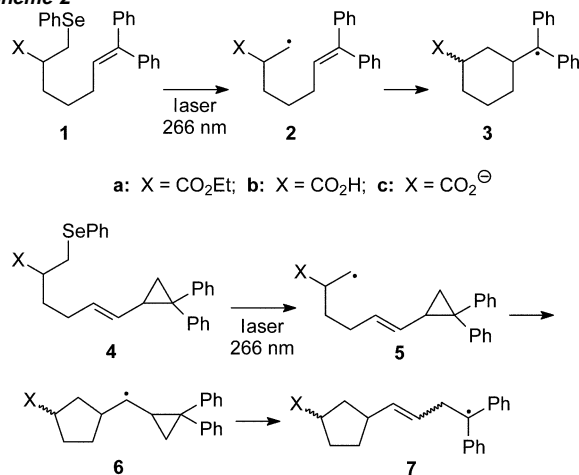


Figure 1. The rearrangement catalyzed by methylmalonyl-CoA mutase, a typical coenzyme B<sub>12</sub>-dependent enzyme-catalyzed rearrangement.

### Scheme 1

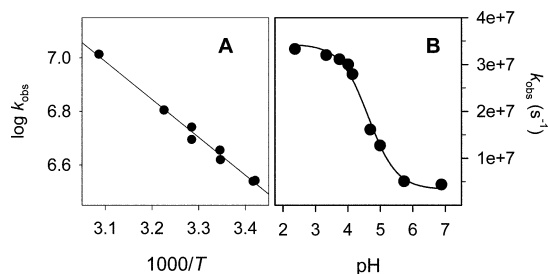


### Scheme 2



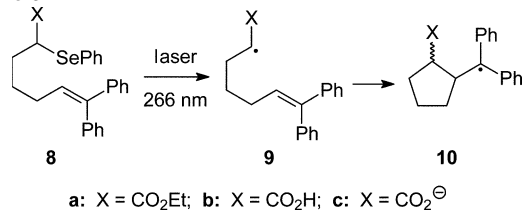
pH 2) and  $5.1 \times 10^6 \text{ s}^{-1}$  (**2c**, pH 11). The 5-*exo* cyclizations had rate constants of  $3.6 \times 10^6 \text{ s}^{-1}$  (**5b**, pH 2) and  $2.8 \times 10^6 \text{ s}^{-1}$  (**5c**, pH 11). For both series, the unsubstituted radicals (**2** and **5**, X = H) cyclize with rate constants of  $5 \times 10^5 \text{ s}^{-1}$ .<sup>12,13</sup> The similar rate constants for the carboxylic acid- and carboxylate-substituted radicals indicate that the kinetic effects are due largely to sterics unlike the case for the  $\alpha$ -substituted radicals below.

Photolysis of the  $\alpha$ -phenylselenenyl ester **8a** gave the known<sup>13</sup>  $\alpha$ -ester radical **9a**, that cyclized to the diphenylalkyl radical **10a** (Scheme 3). The rate constant for cyclization of **9a** in acetonitrile at 22 °C was  $k = 4 \times 10^7 \text{ s}^{-1}$ , which agrees with the value found



**Figure 2.** (A) Arrhenius plot for cyclization of radical anion **9c** in acetonitrile–water (1:1, v:v) at pH 7.4. The function is  $\log k = (11.4 \pm 0.4) - (6.5 \pm 0.6)/\theta$ , where errors are at  $2\sigma$ , and  $\theta = 2.3RT$  in kcal/mol. (B) Kinetic titration of  $\alpha$ -carboxylic acid radical **9b** and radical anion **9c** in water–acetonitrile (1:1, v:v).

### Scheme 3



when **9a** was produced from a different precursor.<sup>13</sup> The  $\alpha$ -phenylselenyl carboxylic acid **8b** behaved similarly, and radical **9b** from photolysis of **8b** also cyclized at 22 °C with a rate constant of  $k = 4 \times 10^7 \text{ s}^{-1}$  in acetonitrile–water (1:1, v:v, pH 2).

Radical anion **9c**, from photolysis of **8c** in acetonitrile–water solution, gave distinctly different results. The rate constant for cyclization of **9c** at 20 °C was  $k = 3.5 \times 10^6 \text{ s}^{-1}$  at pH 7.4, an order of magnitude smaller than the rate constant for cyclization of **9b**. The pronounced effect of the carboxylate group in **9c** is noteworthy because the identity of the X group in other radicals **9** has little effect on the rates of cyclization; for radicals **9** with X = H, CH<sub>3</sub>, OCH<sub>3</sub>, CO<sub>2</sub>Et, CONEt<sub>2</sub>, and CO<sub>2</sub>H, the rate constants for cyclization at 22 °C are in the range  $k = (2\text{--}5) \times 10^7 \text{ s}^{-1}$ .<sup>13,14</sup> A temperature-dependent kinetic study (Figure 2A) showed that the reduction in the rate constant for cyclization of radical anion **9c** is due to an enthalpy effect in the transition state that slows the reaction, even though the entropy of activation for the reaction is more favorable than those for cyclizations of analogues with other X groups. Specifically, the  $E_a$  for cyclization of radical anion **9c** at pH 7.4 of 6.5 kcal/mol is ca. 3 kcal/mol greater than that found for any other radical **9** thus far studied. We speculate that charge delocalization in radical anion **9c** and subsequent localization upon cyclization to **10c** is an important enthalpy feature.

The significant difference in rate constants for cyclizations of radical **9b** and radical anion **9c** permitted a kinetic titration study that demonstrated the robust nature of the approach at various pH (Figure 2B). Reactions were conducted in buffered acetonitrile–water solutions, and the observed rate constants were fit to eq 1, where  $k_A$  and  $k_B$  are rate constants for the cyclization of the acid (**9b**) and basic (**9c**) forms, respectively, and  $K_a$  is the acidity constant for **9b**. Regression analysis gave  $k_A = (3.43 \pm 0.08) \times 10^7 \text{ s}^{-1}$ ,  $k_B = (3.4 \pm 1.0) \times 10^6 \text{ s}^{-1}$ , and  $K_a = (2.3 \pm 0.4) \times 10^{-5}$ , which

generate the line shown in Figure 2B. The apparent  $pK_a$  of  $\alpha$ -carboxylic acid radical **9b** in water is 4.6 (see Supporting Information). The  $\alpha$ -radicals from small alkanic acids have  $pK_a$  values similar to those of the parent acids.<sup>9c</sup>

$$k_{\text{obs}} = (k_A[\text{H}^+] + k_B K_a)(K_a + [\text{H}^+])^{-1} \quad (1)$$

Access to  $\alpha$ - and  $\beta$ -carboxylate radicals could be important for studies of rearrangements catalyzed by coenzyme B<sub>12</sub>-dependent enzymes because one can produce model radical anions that closely resemble the reactive species in nature. The large effect of the carboxylate group in the kinetic parameters for cyclization of **9c**, a reaction that might be expected to have low sensitivity to charge effects, suggests that neutral ester and carboxylic acid radicals might be poor models for the radical anions. We speculated that the negative charge in the radical anions is a critically important feature in rearrangements catalyzed by coenzyme B<sub>12</sub>-dependent enzymes, one that permits heterolytic fragmentation reaction pathways that cannot be accessed from neutral radicals.<sup>6</sup> That speculation can be tested. It is also possible that  $\alpha$ - and  $\beta$ -carboxylate radicals will find applications in synthesis that are patterned after the biological conversions.

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**Supporting Information Available:** Synthetic details and representative LFP results (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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