Supporting Information

Radical Intermediates in Monooxygenase Reactions of Rieske Dioxygenases

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Supplemental Methods

Chemicals. Ethyl acetate (99+ % HPLC grade) and NADH were purchased from Sigma-Aldrich, and anhydrous magnesium sulfate was obtained from Fisher Scientific. NADH was lyophilized overnight to deplete the ethanol in the preparation. Norcarane and bicyclohexane were synthesized as previously described. [1-3]

Growth and Purification of NDO Components. NDO, NDR, and NDF were purified from P*seudomonas species* NCIB 9816-4 as previously described. [4]

Enzyme Reactions. The reactions were performed in a Teflon sealed screw-top vials at 23 °C. A typical reaction contained 25 μM NDO and NDR along with 250 μM NDF in 50 mM MES buffer pH 6.9 plus 100 mM NaCl. 2 - 4 μL of the substrates (**1** or **6**) were added to the side wall of the reaction vial with a gastight syringe, mixed into the solution, and incubated for 30 min prior to the initiation of the reaction. The reaction was initiated by NADH (300 μM final concentration). The final volume of the reaction mixture was 500 μL. The reaction was allowed to proceed for 30 min at 23 °C and then quenched with equal volume of ethyl acetate for product extraction. The quenched solution was vortexed for 1 min and centrifuged for 3 min at 15,000 rpm. The organic layer was removed and passed through a bed of anhydrous magnesium sulfate to remove any aqueous layer. The process was repeated with 250 μL ethyl acetate on the same reaction mixture. The extracted product was stored at –20 °C for later analysis by GC/MS.

Products from norcarane and bicyclohexane oxidation reactions. The expected products from the oxidation of norcarane and bicyclohexane are shown in Figure S1 and S2, respectively.[1-3]

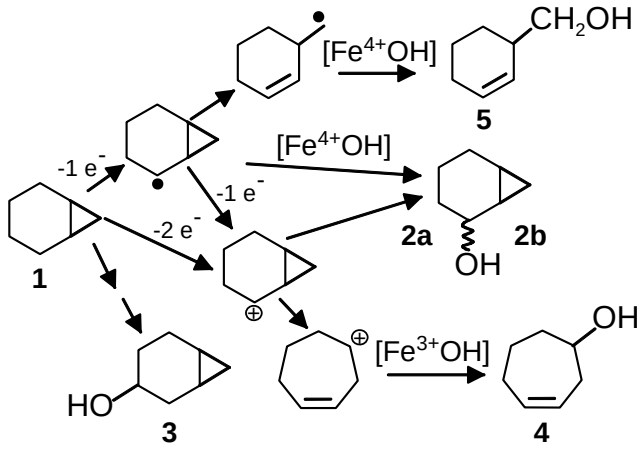


Figure S1. Products expected from oxidation of norcarane. All of these products have been observed from oxygenase enzymes of various types but every enzyme does yield all of these products.

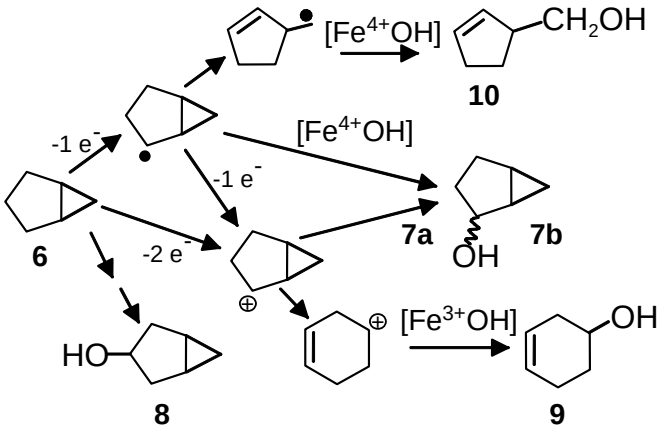


Figure S2. Products expected from the oxidation of bicyclohexane.

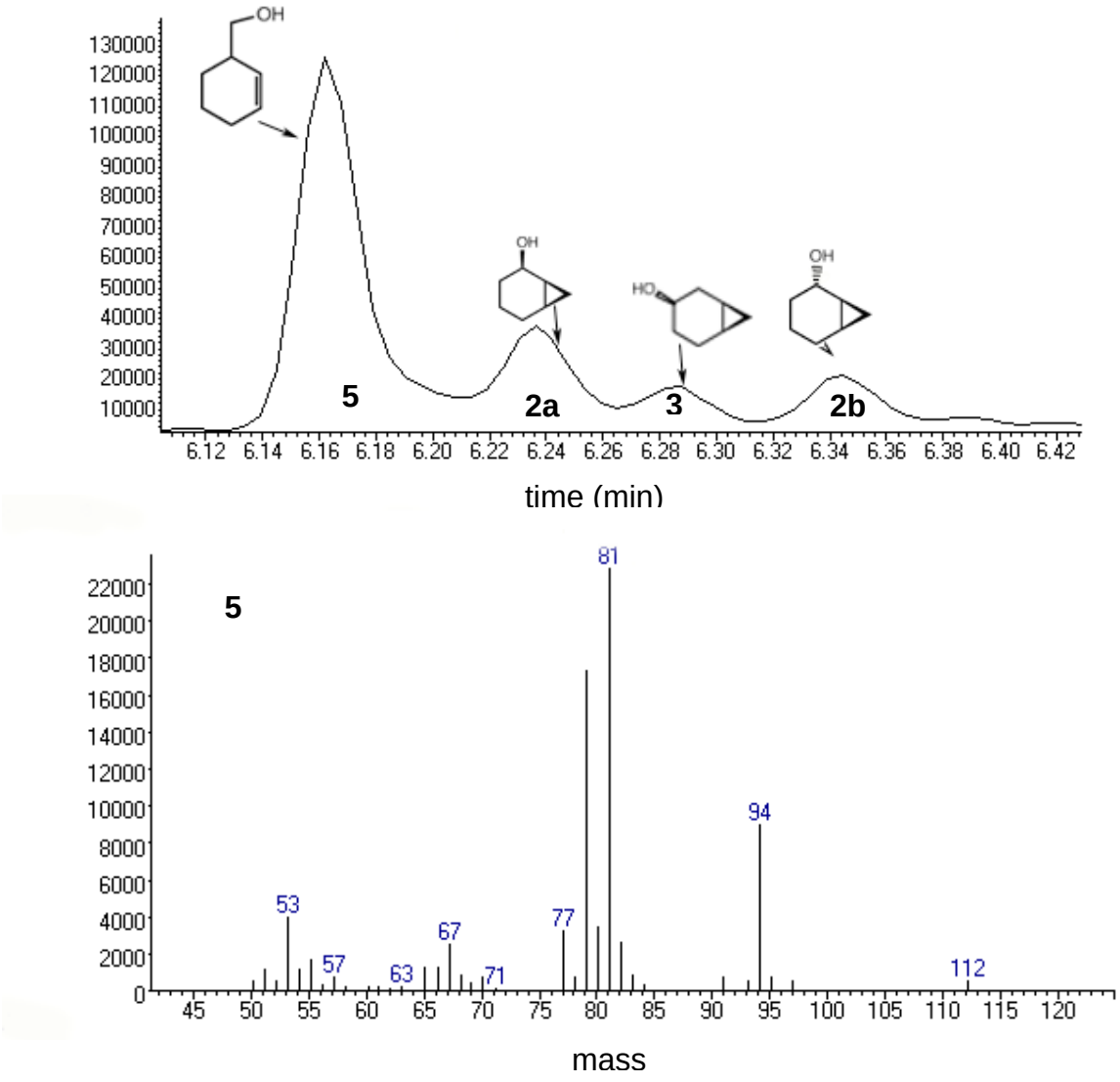
Sample Analysis. Spectra were analyzed on an HP GC 6890/MS 5973 with a HP-5MS cross-linked 5% PH ME Siloxane capillary column (30 m x 0.25 mm x 0.25 μm) run with an initial oven temperature of 50 ºC, ramping to 220 ºC at a ramp rate of 10 ºC/min. For confirmation and higher resolution, some samples were also analyzed using a Shimadzu QP2010 GC/MS equipped with a 0.25 mm x 30.0 mm Supleco SPB-624 capillary column. Authentic products were synthesized and their retention times and fragmentation patterns compared to those of the identified peaks in the GC-MS spectra.[3] Areas under each peak were assumed to be proportional to concentration and were checked as appropriate with concentration standards. Individual mass spectra are obtained by averaging over the peak and relative intensities of key ions do not change over the time course of the peak. For norcarane, the key diagnostic product, hydroxymethylcyclohexene (**5**), has a particularly unique mass spectrum with m/e 81> m/e 79 over the course of the peak, as shown in the supplemental data below. Single and multiturnover data had the same distribution of products and were analyzed in the same way. All authentic standards of the oxidation products of norcarene (the product that occurs when norcarane is desaturated) have also been synthesized and characterized. Under the conditions of our assay, all of these compounds separate with baseline resolution from all other norcarane oxidation products and have distinctly different fragmentation patterns.[5] None of these products are present in any of the NDO oxidations.

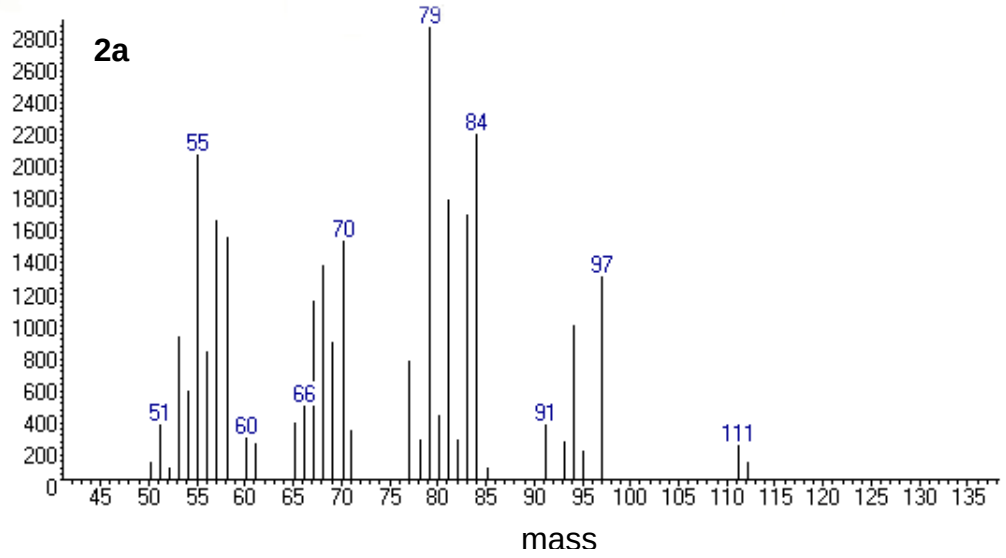
Determination of Radical Intermediate Lifetimes. Lifetimes for the substrate-based radical created in the initial step of the rebound mechanism can be calculated using the following formula: [6]

$$\text{radical lifetime} = \frac{1}{k_{\text{rearrangement}} \dfrac{[\text{ring closed}]}{[\text{ring opened}]}}$$

The $k_{\text{rearrangement}}$ values determined for **1** and **6** are 2×10^8 and 2.9×10^7 s^{-1}, respectively.

Figure S3. GC chromatogram from GC-MS analysis of a characteristic norcarane oxidation catalyzed by NDO with corresponding fragmentation patterns.

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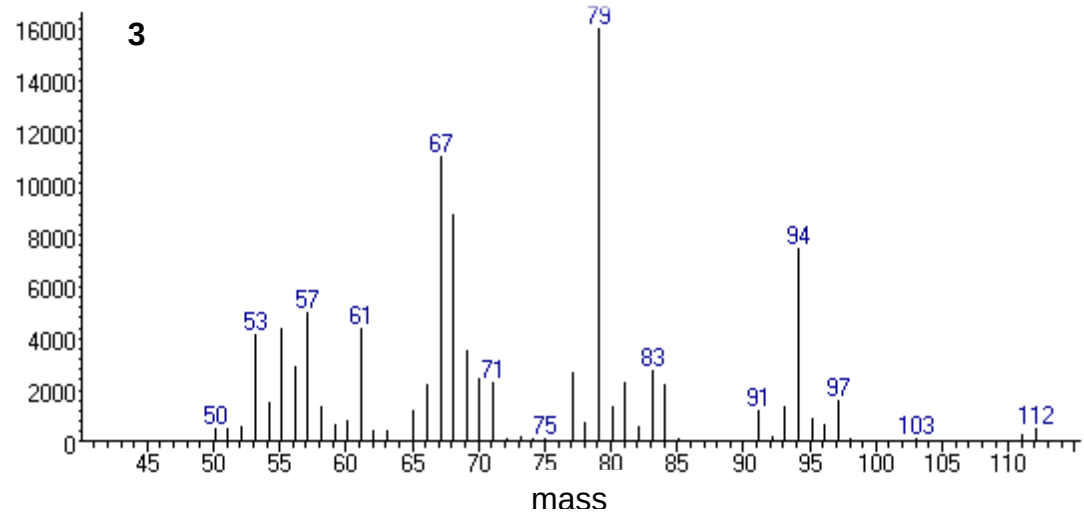
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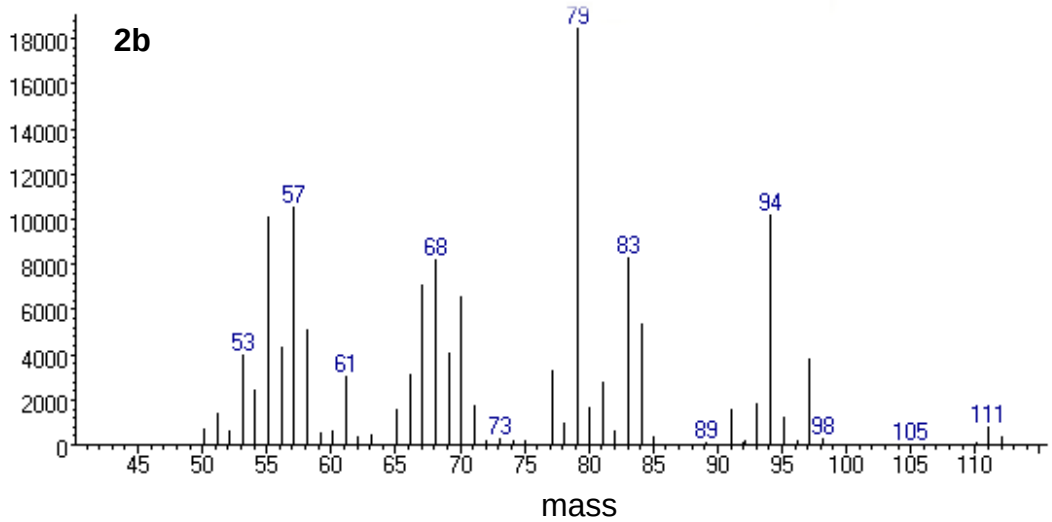
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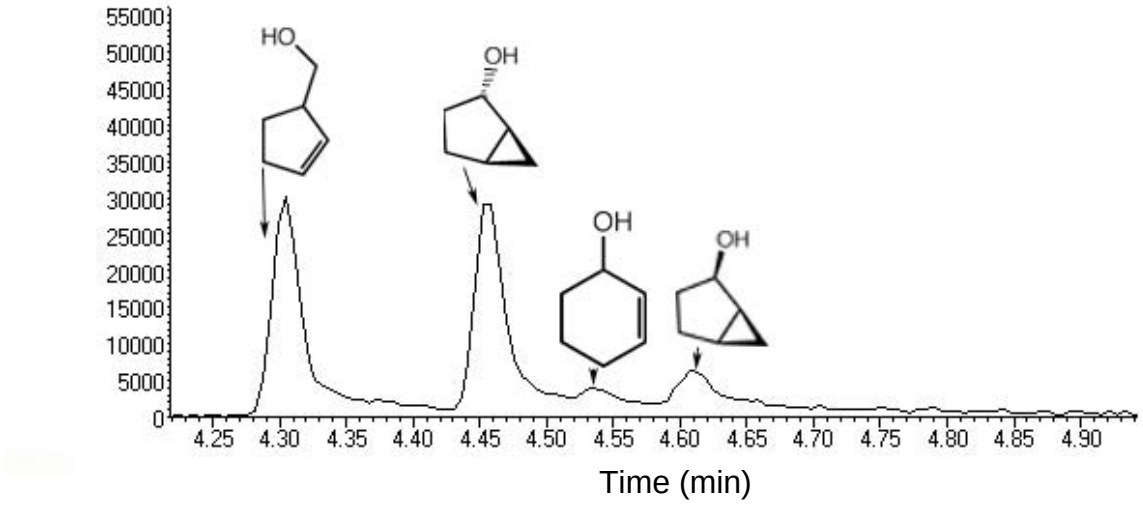

Figure S4. GC chromatogram for GC-MS analysis of a characteristic bicyclohexane oxidation catalyzed by NDO.

References

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