Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

Cage Escape Competes with Geminate Recombination during Alkane Hydroxylation by the Diiron Oxygenase AlkB

Rachel N. Austin, Kate Luddy, Karla Erickson, Marilla Pender-Cudlip, Erin Bertrand, Dayi Deng, Ryan Buzdygon, Jan B. van Beilen, John T. Groves**

Supporting material

Syntheses of substrates and authentic products

Bicyclo[4.1.0]heptane (norcarane), bicyclo[3.1.0]hexane, cyclohex-2-enyl methanol, cyclopent-2-enyl methanol, cyclopent-2-ene carboxaldehyde, 2- and 3-norcaranols, 3-cycloheptenol, cyclohex-3-en-1-ol, bicyclo[3.1.0]hex-2-ene, bicyclo[3.1.0]hexan-2-ols, bicyclo[3.1.0]hexan-3-ols, bicyclo[3.1.0]hexan-3-one and cyclohex-3-en-1-one were prepared according to literature procedures as we have previously described.[1, 2] Bicyclopentane was prepared by the method of Gassman and Mansfield[3]. 2- and 3-Cyclopentenones are commercially available. Bicyclopentan-2-ol was identified by comparison with a published spectrum[4].

3-Norcarene was synthesized by Simmons-Smith reaction on 1,4-cyclohexadiene according to a published procedure[5]. Epoxidation of 3-norcarene with *m*-CPBA, followed by epoxide ring opening with $Li(NEt_2)$ afforded bicyclo[4.1.0]hept-4-en-3-ol[6]. 2-Norcarene was prepared by Simmons Smith reaction on 1,3-cyclohexadiene. Bicyclo[4.1.0]hept-3-en-2-ol was synthesized by the Diels-Alder reaction of cyclopropene with 1,3-butadiene-1-trimethylsilyl ether followed by deprotection in HCl/CH_3OH. The structure of the product was confirmed by comparison with known spectra[7].

Whole cell and cell free extract experiments

Substrate oxidations with whole cells and cell-free extracts were performed as we have previously described.[1]

Mass spectral data collection and analysis:

Spectra were obtained on either a 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) or a high sensitivity Shimadzu GCMS-QP2010 equipped with a 0.25 mmX30.0 m, Supelco SPB-624 capillary column. With the HP GC 6890, the samples were run with an initial oven temperature of 50 ºC, ramping to 250 ºC at a ramp rate of 10 ºC/min. For bicyclohexane samples where 3-bicyclohexanol coeluted with 2-endo-bicyclohexanol under the standard conditions, the initial oven temperature was 50 ^{o}C and the temperature was held there for 20 min before ramping to 250 ºC at 10 ºC/min**.** Areas under each peak in the TIC traces were taken to be proportional to concentration and were checked as appropriate with concentration standards. Authentic products were prepared as described above and their retention times and fragmentation patterns compared to those of the identified peaks in the GC-MS spectra.

Small amounts of cyclohex-2-enecarboxylic acid and cyclopent-2-enecarboxylic acid were identified in several experiments. These were initially identified in control experiments where the corresponding alcohols were used as substrates for AlkB. They were confirmed by comparison to the authentic standards that were synthesized according to published procedure[8]. The small amounts of these products detected had no effect on the ratio of ring opened to ring closed products. Both 2- and 3-norcarene and very small amounts of the corresponding oxidation products were occasionally detected but since the amount of these products was very small and since the authentic 2- and 3-norcarane

oxidation products did not coelute with any of the hydroxylated products being analyzed under our conditions, they did not interfere with the analyses presented here.

Control experiments

Sterile control experiments were performed for all *in vivo* experiments where all components were added to sterilized media and incubated at the same temperature and for the same length of time as the live experiments. Control experiments were done for the cell free experiments by adding all substrate and NADH to buffer and incubating the vial at the same temperature and for the same length of time as the live experiments.

GC-MS data for the oxygenation of the various substrates by AlkB.

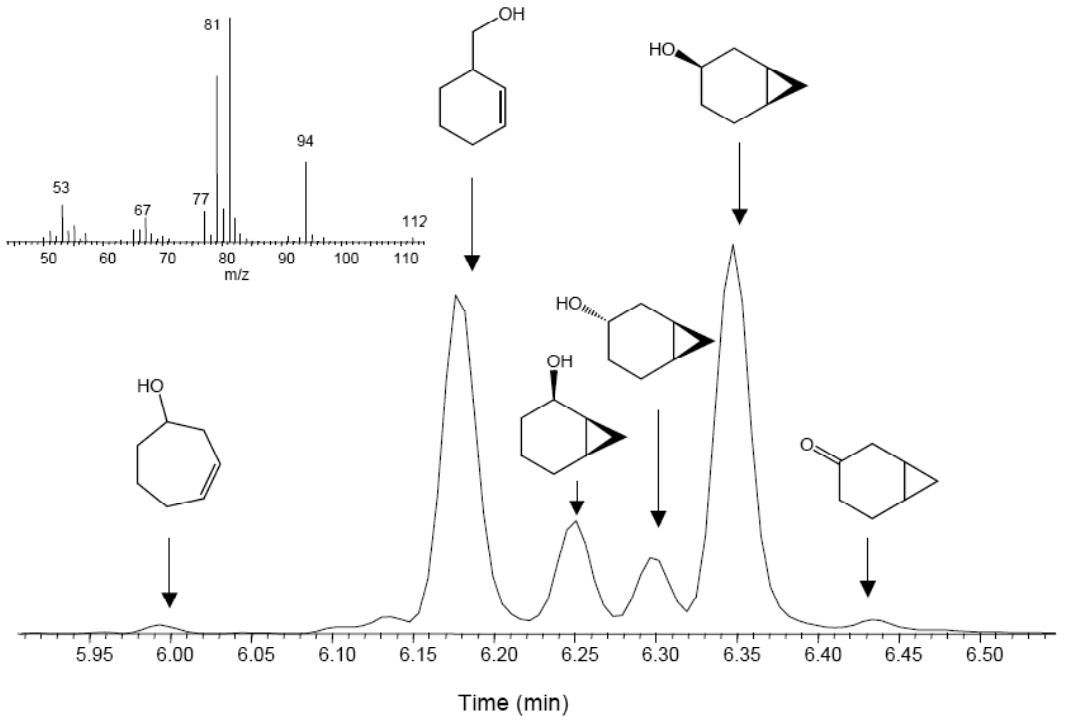


Figure S1. Product mixture for norcarane oxygenation by AlkB (cell free extract). Inset: Highly characteristic mass spectrum of cyclohex-2-enyl methanol (6.18 min), which was invariant over the course of the peak.

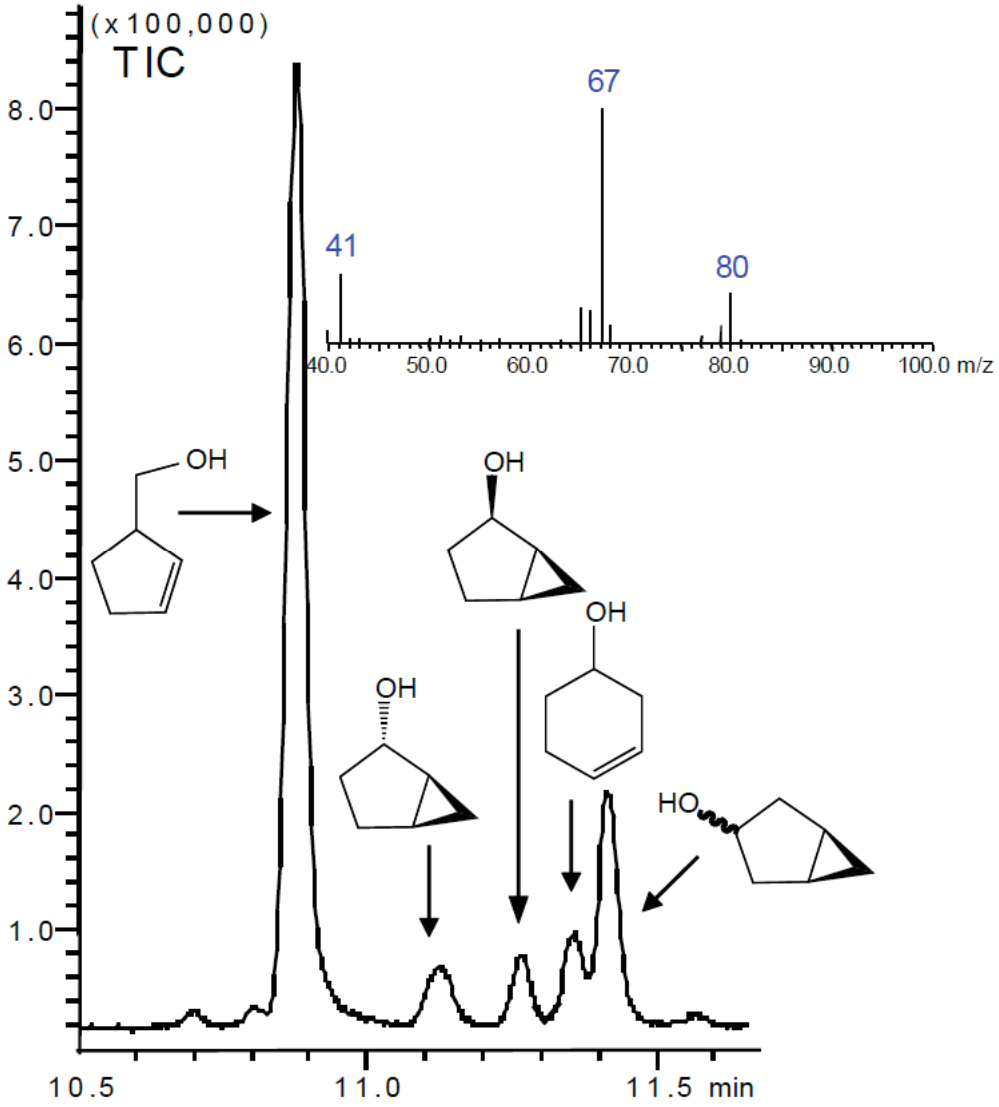


Figure S2. Product mixture for bicyclo[3.1.0]hexane oxygenation by AlkB in GPo1, whole cell method. Inset: Mass spectrum of cyclopent-2-enyl methanol (10.9 min).

Mixed substrate experiments - bicyclohexane and norcarane

Experiments were performed in which equal amounts of norcarane and bicyclohexane were used as substrates. Data from these experiments is presented in Table S1. The values of R/U obtained were consistent with those in Table 1 obtained with pure substrates.

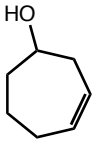
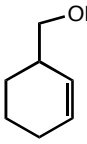
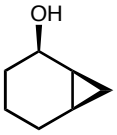
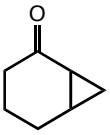
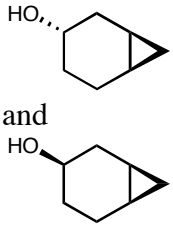
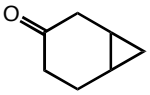
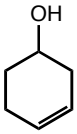
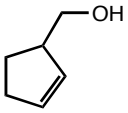
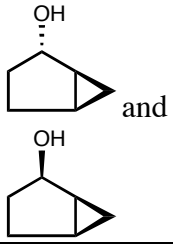
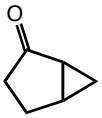
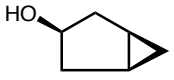
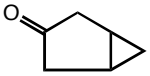
							R/U
GPo1	0	17.2	12.6	0	70.2	0	1.38
							R/U
GPo1	9.4	62.4	15.1	0	13.1	0	4.1

Table S1. Distribution of products from GPo1 whole cell oxygenation of 1:1 norcarane/bicyclohexane.

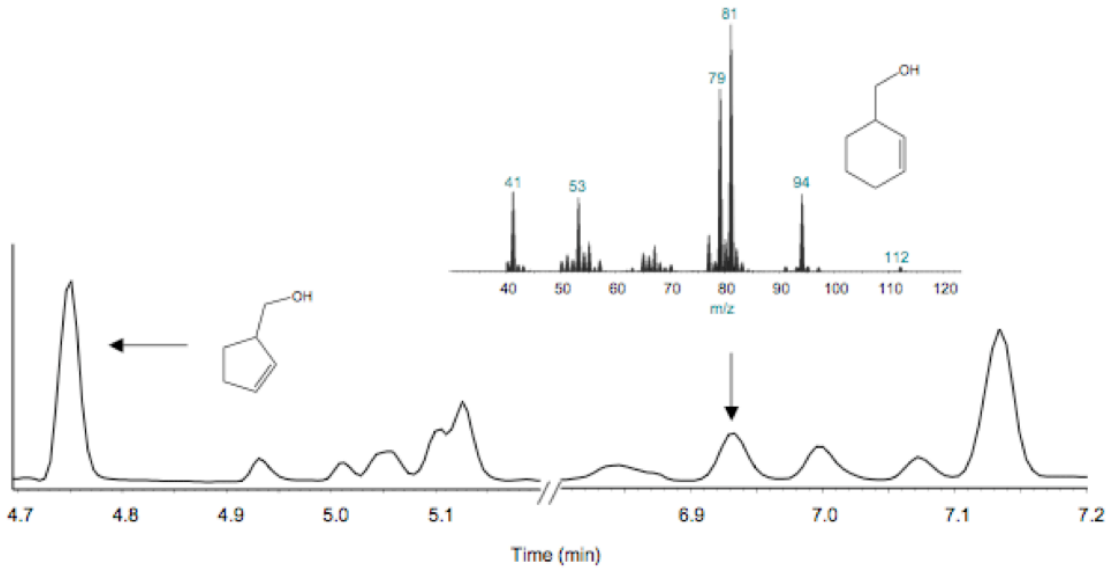


Figure S3. GC-MS (TIC) of product mixture from whole cell oxygenation of 1:1 norcarane/bicyclohexane. Inset: Mass spectrum cyclohex-2-enyl methanol.

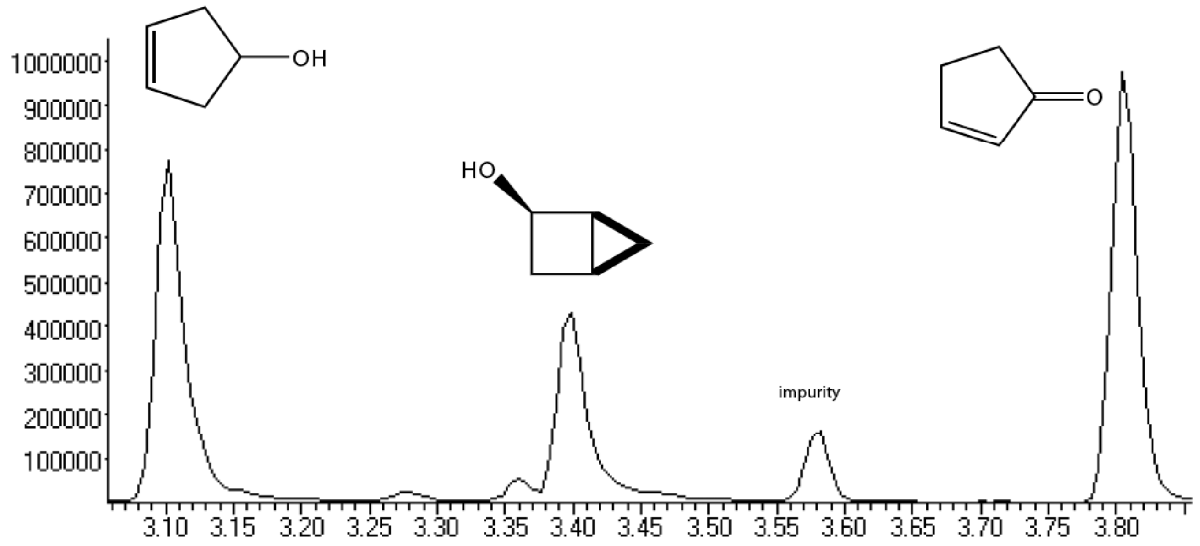


Figure S4. GC-MS (TIC) of product mixture from AlkB oxygenation (GPo1 whole cell) of bicyclo[2.1.0]pentane.

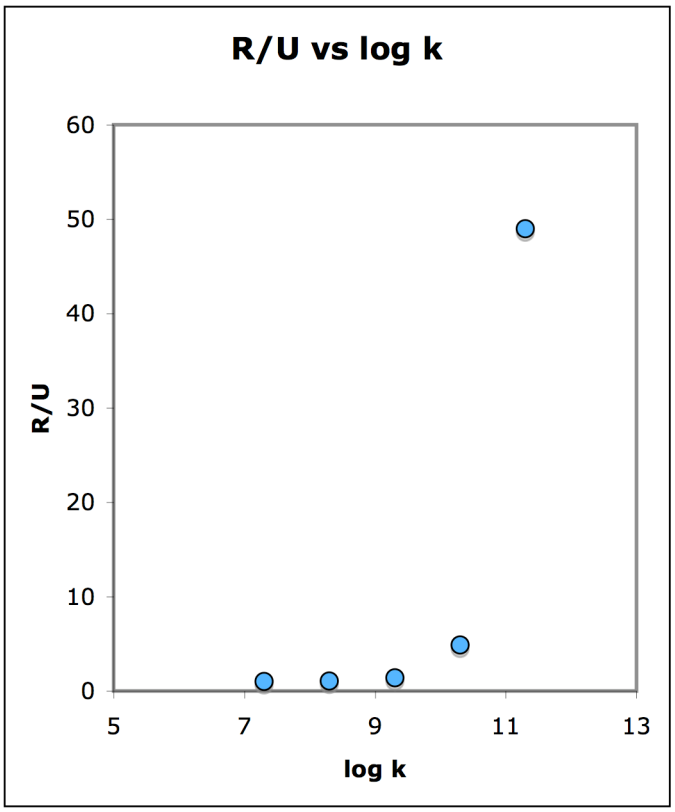


Figure S5. Plot of the calculated ratio of rearranged and unrearranged product alcohols (R/U) as a function of the rearrangement rate constant k_{rearr}. Values were determined by numerical simulation of the reactions in Scheme 2 with $k_R = k_e = 5 \times 10^9$ s^{-1}. The value of k_{rearr} was varied over the range of the substrates used, 2×10^7 s^{-1} to 2×10^{11} s^{-1}. As can be seen, R/U is calculated to be near unity for the slower rearranging bicycloalkane substrates (1.0, 1.04 and 1.38) while phenyl-methylcyclopropane, with $k_{rearr} = 2 \times 10^{11}$ s^{-1}, is predicted to be fully rearranged (R/U = 49) as was experimentally observed.

[1] R. N. Austin, D. Deng, Y. Jiang, K. Luddy, J. B. van Beilen, P. R. Ortiz de Montellano, J. T. Groves, *Angew. Chem. Int. Ed.* **2006**, *45*, 8192.
[2] E. Rozhkova-Novosad, J.-C. Chae, G. J. Zylstra, E. M. Bertrand, M. Alexander-Ozinskas, D. Deng, L. A. Moe, J. B. van Beilen, M. Danahy, J. T. Groves, R. N. Austin, *Chem. Biol.* **2007**, *14*, 165.
[3] P. G. Gassman, K. T. Mansfield, *Org. Synth.* **1969**, *49*, 1.
[4] P. R. Ortiz de Montellano, R. A. Stearns, *J. Am. Chem. Soc.* **1987**, *109*, 3415.
[5] E. C. Friedrich, *J. Org. Chem.* **1969**, *34*, 528.
[6] L. A. Paquette, W. E. Fristad, C. A. Schuman, M. A. Beno, G. G. Christoph, *J. Am. Chem. Soc.* **1979**, *101*, 4645.
[7] M. Newcomb, D. S. P. Lansakara-P, H. Y. Kim, R. E. P. Chandrasena, S. J. Lippard, L. G. Beauvais, L. J. Murray, V. Izzo, P. F. Hollenberg, M. J. Coon, *J. Org. Chem.* **2007**, *72*, 1128.
[8] K. Jones, R. F. Newton, C. Yarnold, *Synth. Commun.* **1992**, *22*, 3089.