Investigating the mechanism of naphthalene 1,2-dioxygenase in whole cells

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Mycobacterium tuberculosis

The alveolar macrophage detects M. tb.
Mechanisms of Alkane Hydroxylation

The ability of heme-containing P450s and non-heme oxygenases to hydroxylate hydrocarbon substrates derives from high-valent iron intermediates, Fe=O.

Related non-heme systems

Naphthalene Dioxygenase (NDO)
A non-heme mono-Fe system

Useful for bioremediation and enantioselective catalysis
There have been many proposals
**In vivo technique**

- Allows proteins to react in their native environments and avoids difficult isolation
- Used in the past with other enzyme systems (e.g. AlkB, P450) and has yielded results in accord with *in vitro* data
- Can easily demonstrate cells are releasing representative samples by lysing cells
- Can demonstrate products aren't reacting further by feeding product mixtures to cells
Bicyclo[4.1.0]heptane (norcarane)

$k_{\text{rear}} = 2 \times 10^8 \text{ s}^{-1} \rightarrow \text{radical lifetime} \approx 8.1 \text{ ns}$
trans-1-methyl-2-phenylcyclopropane (PMCP)

\[ k_{\text{rear}} \approx 10^{11} \text{ s}^{-1} \rightarrow \text{radical} \]

lifetime \approx 15 \text{ ps}

Rearranged alcohol 60.1
Unrearranged alcohol 39.9
Conclusions

- Variety of diagnostic probes hydroxylated in mechanistically informative positions with no over-oxidation or cationic products
- Norcarane, bicyclohexane, and DECP intermediate lifetimes agree well; TMCP and PMCP intermediate lifetimes agree well → 1º vs. 2º carbon effect?
- Various amounts of $^{18}\text{O}$ in products → Fe(V)-oxo-oxo-hydroxo species formed but its reactivity is substrate dependent