Evidence for Enzymatic Catalysis of the Diels-Alder Reaction in Nature

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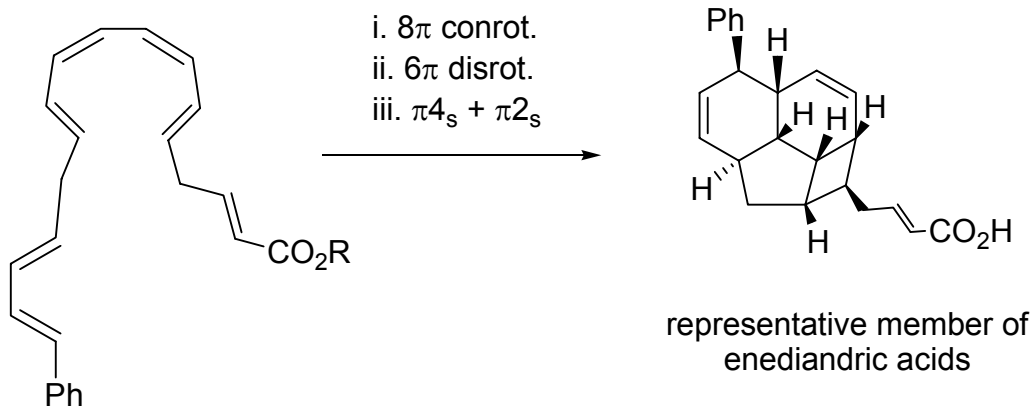
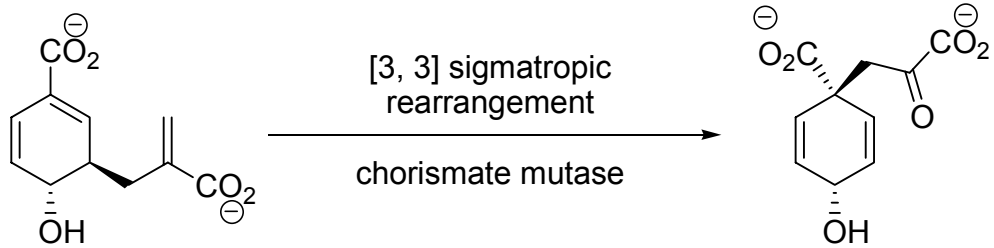
Sorensen Group
Organic Supergroup Literature Presentation
July 2005

Does Nature Know the Diels-Alder Reaction?

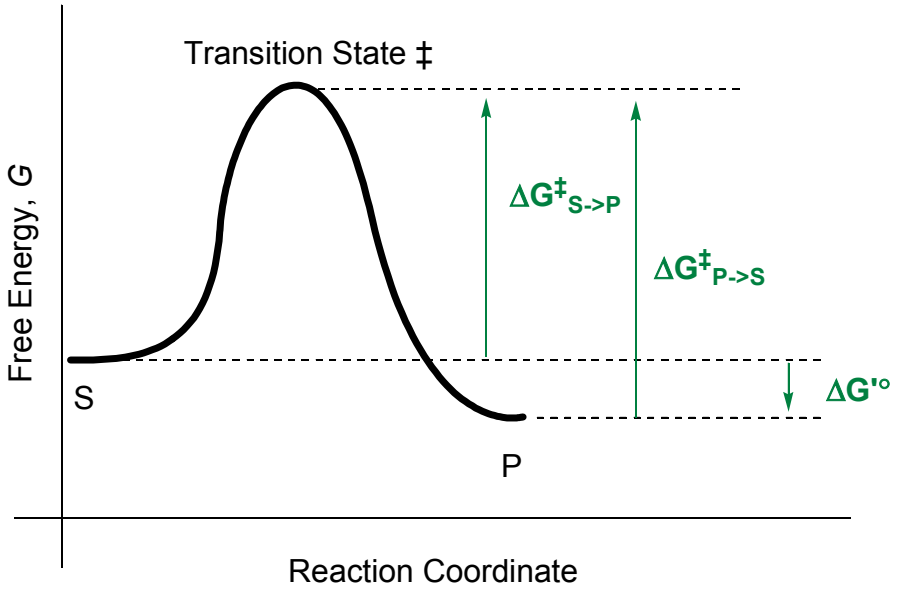


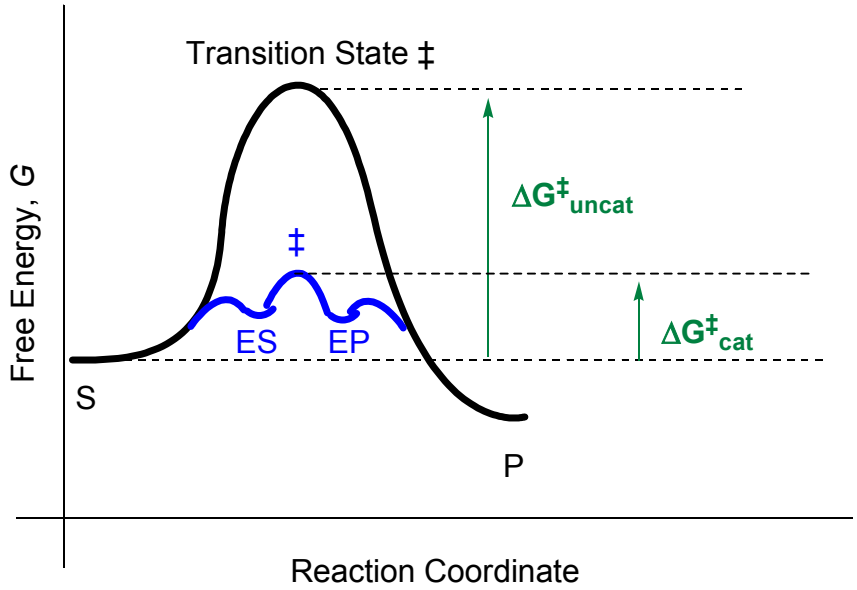
representative member of enediandric acids



Laschat, S. *Angew Chem. Int. Ed.* **1996**, *35*, 289.
Nicolaou, K. C.; Sorensen, E. J. in Classics in Total Synthesis, **1996**, 265-267.

What is an Enzyme? Thermodynamic Review





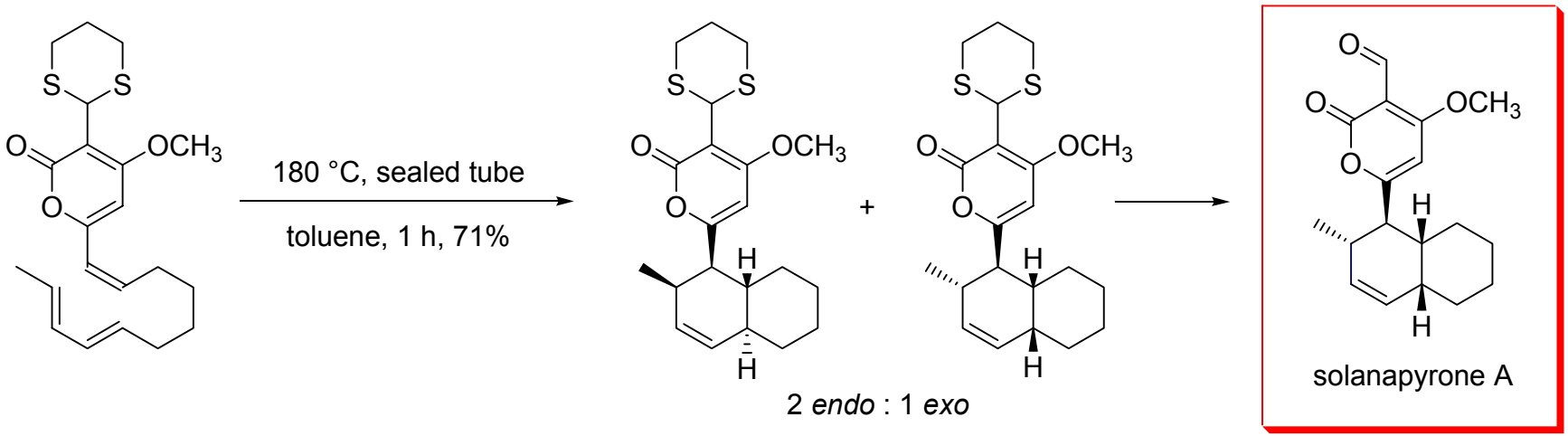
$$\Delta G'^{\circ} = -RT \ln K'_{eq}$$

$$k = Ae^{-\Delta G‡/RT}$$

Catalysts do NOT affect reaction equilibria.
Catalysts enhance reaction rates by lowering activation energies.

Nelson, D. L.; Cox, M. M. in Lehninger Principles of Biochemistry, 3rd ed. **2000**, 246-250.

Solanapyrone A



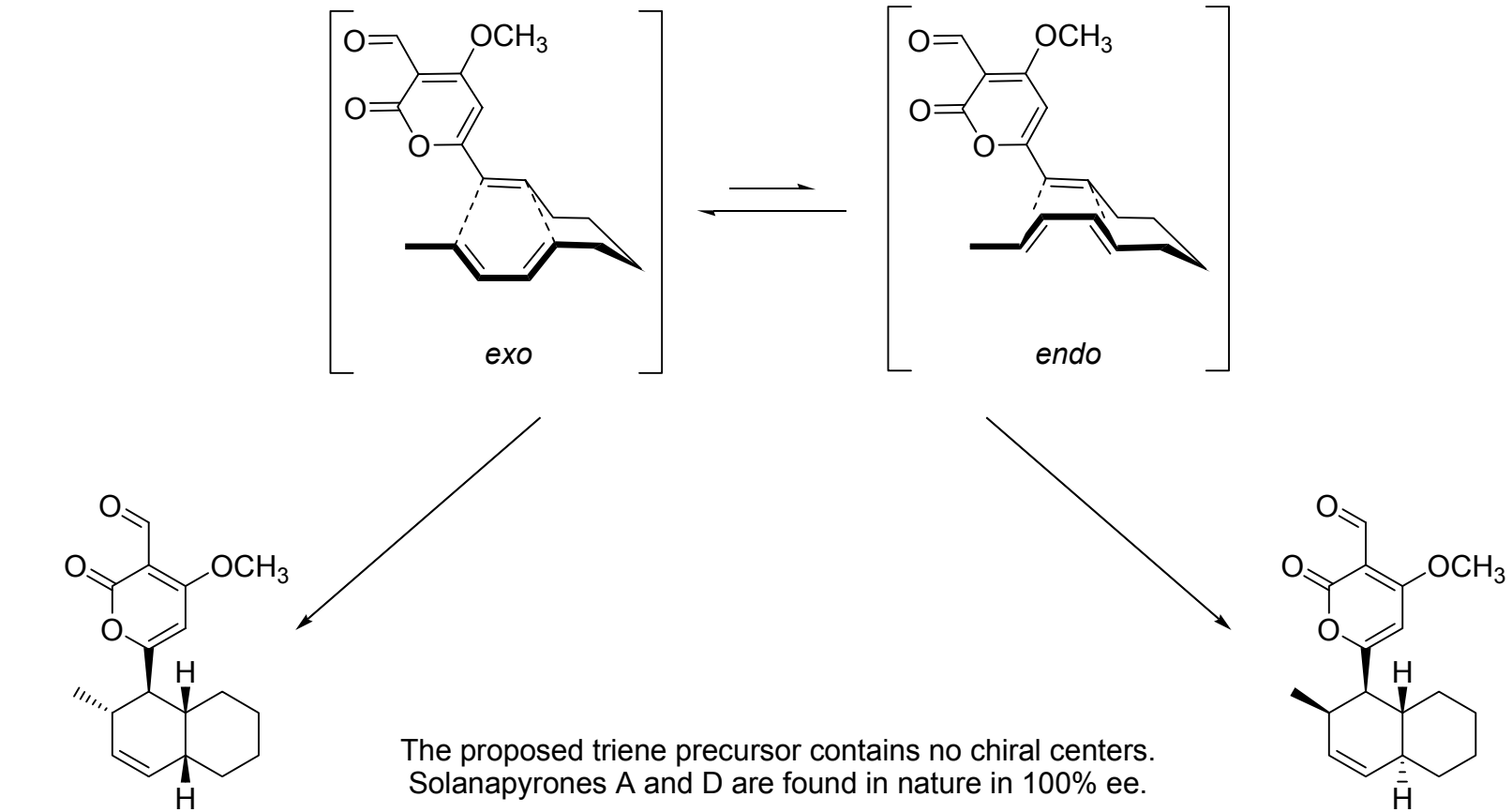
Solanapyrone A: decalin polyketide phytotoxin produced by the pathogenic fungus *Alternaria solani*, causal organism of potato early blight disease

Solanapyrone A inhibits DNA polymerase β and γ.

The first synthesis of (±)-solanapyrone A was achieved through an intramolecular Diels-Alder reaction, which fueled speculation about its assembly in Nature.

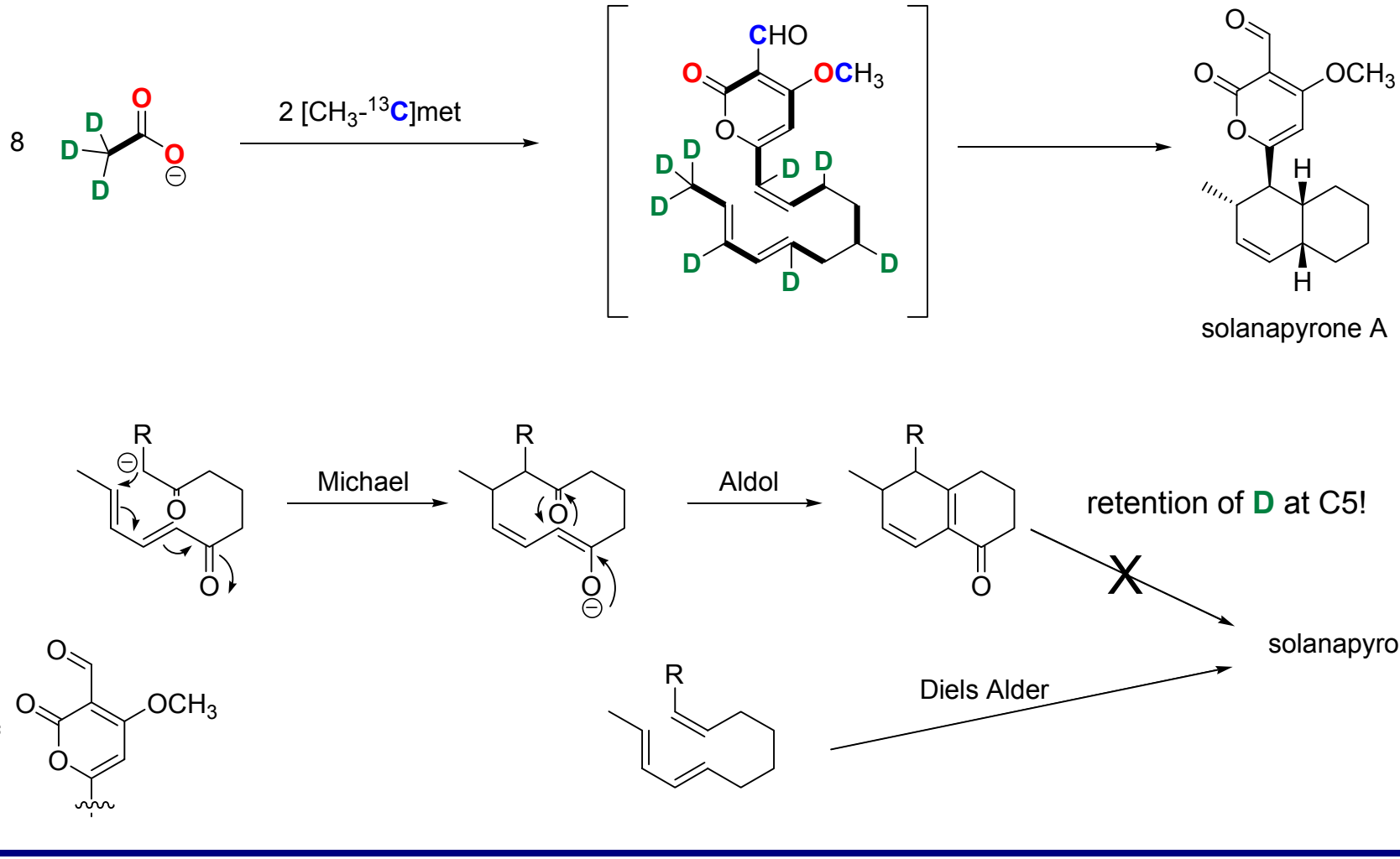
Ichihara, A.; Tazaki, H.; Sakamura, S. *Tet. Lett.* **1983**, *24*, 5373
Ichihara, A.; Miki, M.; Tazaki, H.; Sakamura, S. *Tet. Lett.* **1987**, *28*, 1175.
Mizushina, Y., et al. *J. Biol. Chem.* **2002**, *277*, 630-638.

Solanapyrone D and a Proposed Biosynthesis



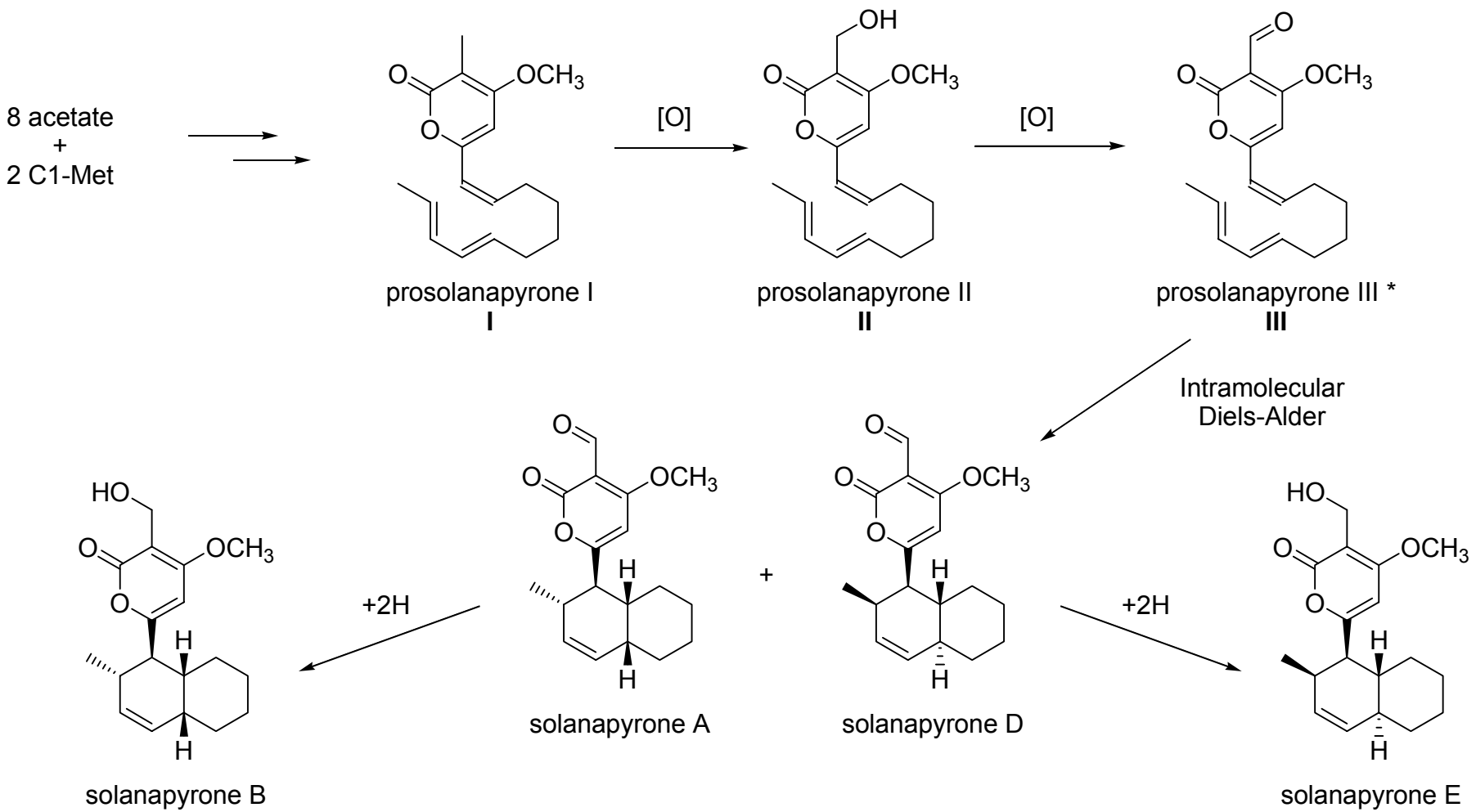
Oikawa, H.; Yokota, T.; Ichihara, A.; Sakamura, S. *J. Chem. Soc. Chem. Comm*. **1989**, 1284.
Oikawa, H.; Suzuki, Y.; Naya, A.; Katayama, K.; Ichihara, A. *J. Am. Chem. Soc.* **1994**, *116*, 3605.

Solanapyrones: Feeding Experiment Summary



Oikawa, H.; Yokota, T.; Abe, T.; Ichihara, A.; Sakamura, S.; Yoshizawa, Y.; Vederas, J. C. *J. Chem. Soc. Chem. Comm.* **1989**, 1282.

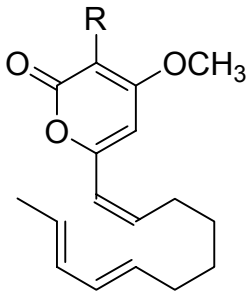
Biosynthesis: Summary of Feeding Experiments



Oikawa, H.; Suzuki, Y.; Naya, A.; Katayama, K.; Ichihara, A. *J. Am. Chem. Soc.* **1994**, *116*, 3605.

* Feeding experiments with **III** inconclusive. **III** underwent spontaneous *endo* cyclization in aqueous conditions.

Solution Reactivity of Prosolanapyrones



R = CH_3 (**I**)

R = CH_2OH (**II**)

R = CHO (**III**)

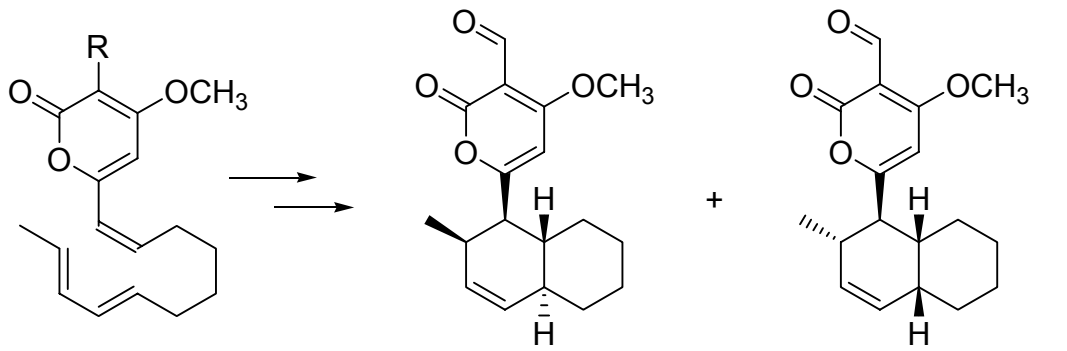
substrate	solvent	temperature (°C)	time (h)	yield (%)	SM recovery (%)	*endo*/*exo*
I	$PhCH_3$	180	48	12	11	1.9
I	H_2O	30	168	7	93	> 10
II	$PhCH_3$	110	48	55	2	2.2
II	$CHCl_3$	110	2	7	91	3.6
II	CH_3CN	110	24	71	18	5.6
II	H_2O	30	48	19	81	20
III	$PhCH_3$	110	1	68	27	2.7
III	$CHCl_3$	110	1	64	28	3.4
III	CH_3CN	110	1	82	10	4.4
III	H_2O	30	3	62	28	23

Endo-selectivity increases with increasing solvent polarity.

Rate depends on the oxidation level of the pyrone substituent.

Oikawa, H.; Kobayashi, T.; Katayama, K.; Suzuki, Y.; Ichihara, A. *J. Org. Chem.* **1998**, *63*, 8748.

Solanapyrone Synthase: Improved *Exo* Selectivity



solanapyrone D (*endo*) solanapyrone A (*exo*)

R = CH_3 (**I**)

R = CH_2OH (**II**)

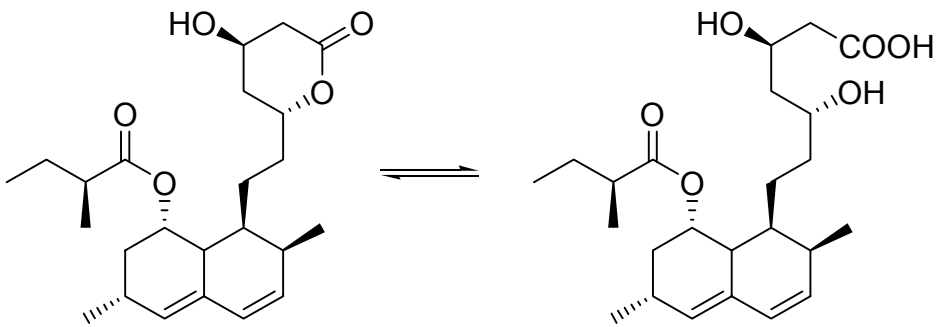
R = CHO (**III**)

substrate	conditions	yield (%)	SM recovery (%)	*endo* :*exo*	ee (%)
II	control	0	100	n/a	
II	+ extract	19	75 + 6 % of III	0.176	99
III	control	15	85	32.3	
III	+ extract	25	75	0.887	92
III	+ denatured extract	10	90	32.3	

Crude enzyme preparation oxidized **II** to **III**. No reaction of **II** in absence of $O_{2(g)}$.
Background uncatalyzed cyclization of **III** competes with enzymatic reaction, hence lower ee when **III** is used as starting material.
Enzyme not yet isolated; further purification of the enzyme(s) responsible is in progress.

Oikawa, H.; Katayama, K.; Suzuki, Y.; Ichihara, A. *J. Chem. Soc. Chem. Comm.* **1995**, 1321.
Katayama, K.; Kobayashi, T.; Oikawa, H.; Honma, M.; Ichihara, A. *Biochim. et Biophys. Acta*, **1998**, 387.
An enantioselective synthesis of solanapyrone A utilized this crude enzyme for the IMDA:
Oikawa, H.; Kobayashi, T.; Katayama, K.; Suzuki, Y.; Ichihara, A. *J. Org. Chem.* **1998**, *63*, 8748.

Lovastatin



lovastatin (closed form) lovastatin (open form) dihydromonacolin L

Lovastatin (mevinolin) is produced by fermentation of the fungal strain *Aspergillus terreus*, and has also been isolated from *Monascus ruber*.

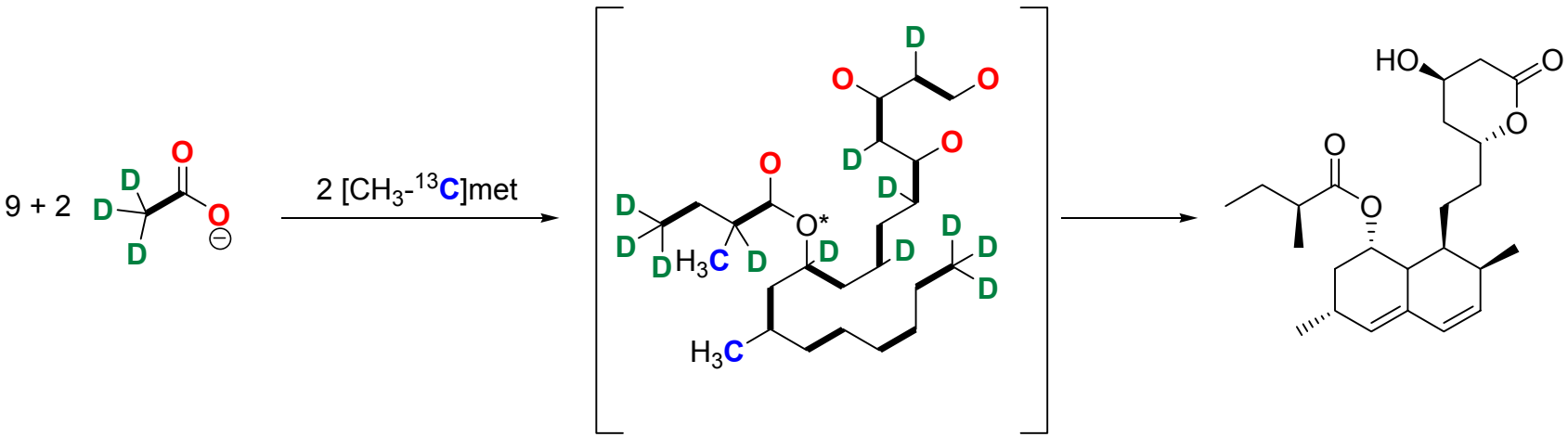
The lactone opened form is a potent inhibitor of the liver enzyme HMG-CoA reductase, which reduces HMG-CoA to mevalonate, the rate limiting step in cholesterol biosynthesis.

Prescribed as Mevacor (Merck) to lower cholesterol and fats in blood.

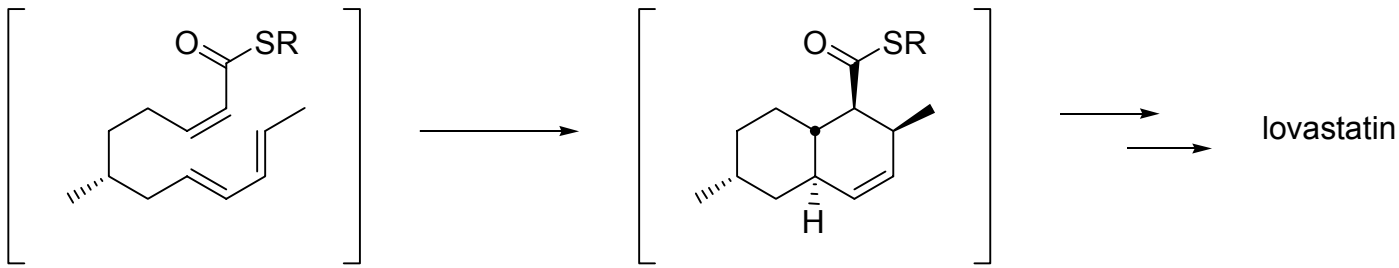
No bicyclic precursor less oxidized than dihydromonacolin L has been reported.

Alberts, A. W., et al. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3957.
Endo, A. *J. Antibiot.* **1979**, *32*, 852.
Endo, A. *Trends Biochem. Sci.* **1981**, *6*, 10.
Kennedy, J.; Auclair, K.; Kendrew, S. G.; Park, C.; Vederas, J. C.; Hutchison, C. R. *Science* **1999**, *284*, 1368.

Lovastatin: Feeding Experiment Summary

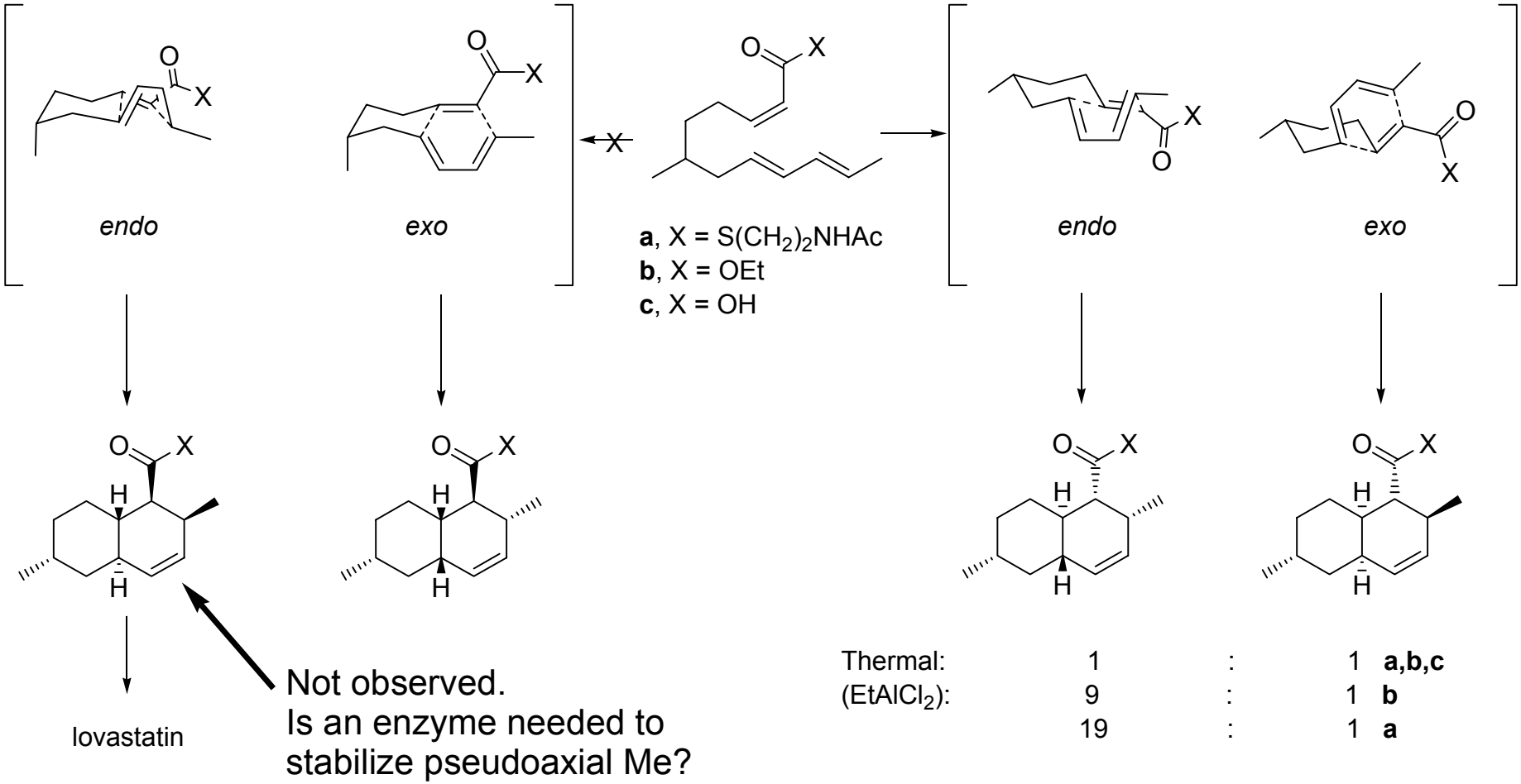


Diels-Alder proposed as key biogenesis step:



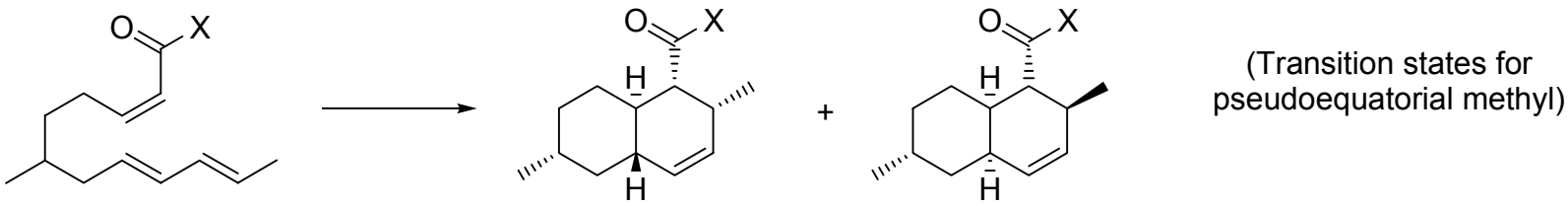
Chan, J. K.; Moore, R. N.; Nakashima, T. T.; Vederas, J. C. *J. Am. Chem. Soc.* **1983**, *105*, 3334.
Moore, R. N.; Bigam, G.; Chan, J. K.; Hogg, A. M.; Nakashima, T. T.; Vederas, J. C. *J. Am. Chem. Soc.* **1985**, *107*, 3694.
Yoshizawa, Y.; Witter, D. J.; Liu, Y. Q.; Vederas, J. C. *J. Am. Chem. Soc.* **1994**, *116*, 2693.

Lovastatin: Attempted Laboratory Cyclizations



Witter, D .J.; Vederas, J. C. *J. Org. Chem.* **1996**, *61*, 2613.

Solution Reactivity of Lovastatin Precursor



(Transition states for pseudoequatorial methyl)

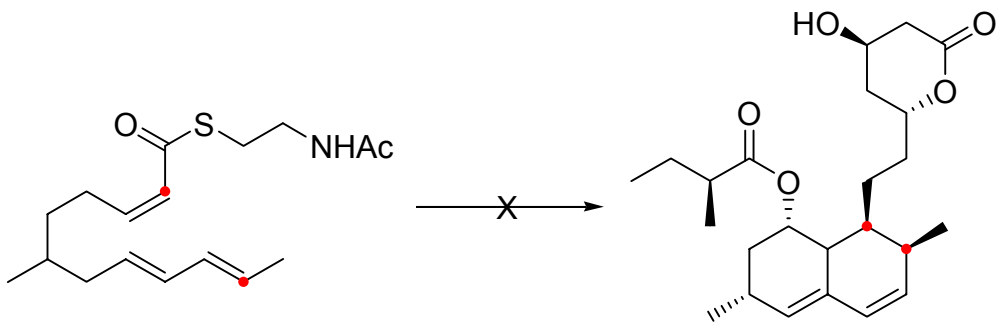
a, X = $S(CH_2)_2NHAc$
b, X = OEt
c, X = OH

endo *exo*

substrate	conditions	temperature (°C)	time (h)	yield (%)	SM recovery (%)	*endo*/*exo*
a	$PhCH_3$	160	96	81	n/a	1
b	$PhCH_3$	160	96	72	6	1
c	$PhCH_3$	160	96	83	n/a	1
a	$EtAlCl_2$ + $PhCH_3$	23	3	80	n/a	9
b	$EtAlCl_2$ + $PhCH_3$	23	3	58	n/a	19
b	$CHCl_3$	22	240	50	n/a	n/a
b	H_2O:CH_3CN:MeOH (5:5:1)	28	48	50	n/a	n/a

Witter, D .J.; Vederas, J. C. *J. Org. Chem.* **1996**, *61*, 2613.

Lovastatin: Attempted Feeding Experiment

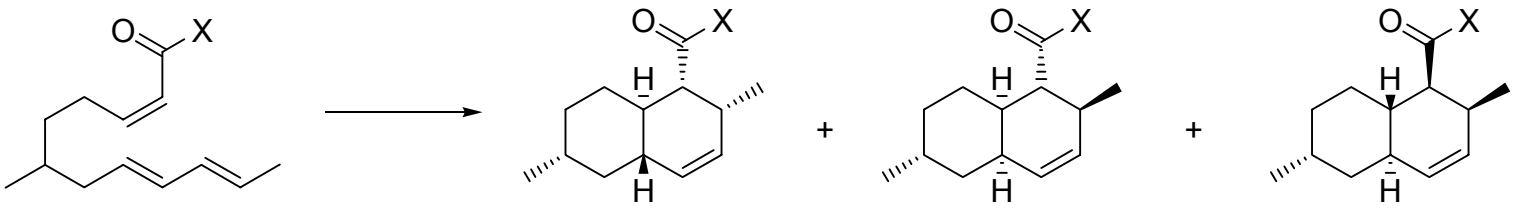


lovastatin (closed form)

Feeding this ^{13}C labeled substrate to *Aspergillus terreus* resulted in no formation of carbon-carbon coupled lovastatin or precursors. Presumably, the substrate is catabolized before it can undergo cycloaddition.

Witter, D .J.; Vederas, J. C. *J. Org. Chem.* **1996**, *61*, 2613.

LNKS Enzyme Affords Correct Stereochemistry



a, X = $S(CH_2)_2NHAc$
b, X = OEt

1

2

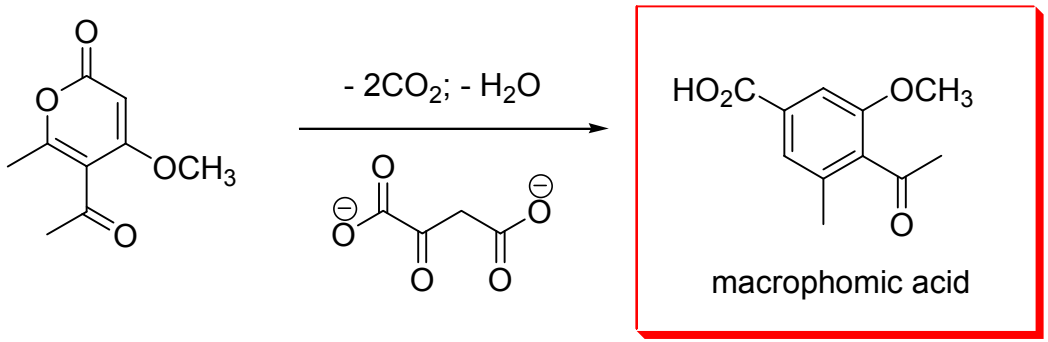
3
natural product's stereochemistry

Stereochemistry found in the natural product (3) is only obtainable in the presence of purified LNKS enzyme. The enzyme may stabilize the transition state through van der Waals or other contacts.

k_{cat} = 0.073 ± 0.001 min^{-1}. Nonenzymatic cyclization competes with catalysis.

Auclair, K.; Sutherland, A.; Kennedy, J.; Witter, D. J.; Van den Heever, J. P.; Hutchinson, C. R.; Vederas, J. C. *J. Am. Chem. Soc.* **2000**, *122*, 11519.

Macrophomate: Benzoate from a Pyrone



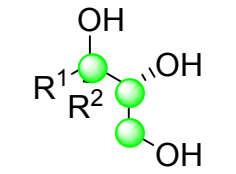
Isolated from *Macrophoma commelinae* fungus, which causes spots on the leaves of the Asiatic dayflower.

The benzoate macrophomate is made by an unusual multistep transformation from a 2-pyrone. This type of aromatic compound is typically biosynthesized *via* the shikimate or polyketide pathway.

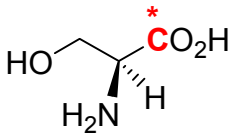
http://www.sycamoreisland.org

Sakurai, I.; Suzuki, H.; Miyaijima, K.; Akiyama, S.; Simizu, S.; Yamamoto, Y. *Chem. Pharm. Bull.* **1985**, *33*, 5141.
Oikawa, H.; Yagi, K.; Watanabe, K.; Honma, M.; Ichihara, A.; *Chem. Commun.* **1997**, 97.

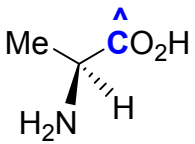
Macrophomate: Summary of Biosynthetic Studies



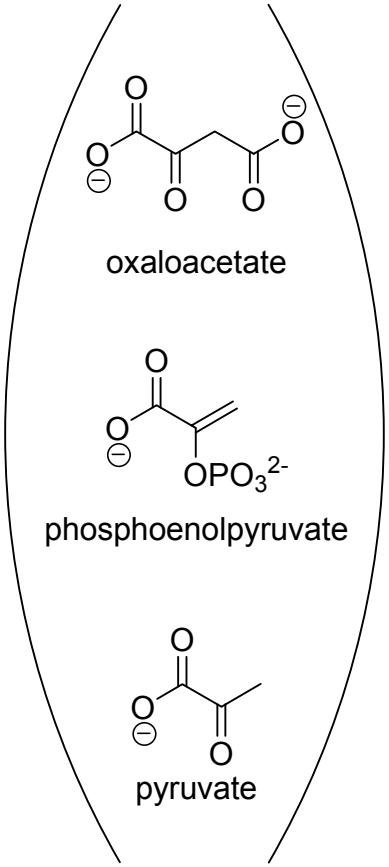
glycerol
R^1, R^2 = H or D



[1-^{13}C]-L-serine



[1-^{13}C]-L-alanine

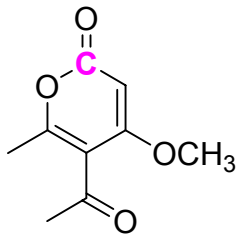


oxaloacetate

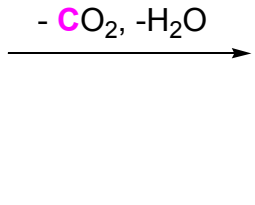
phosphoenolpyruvate

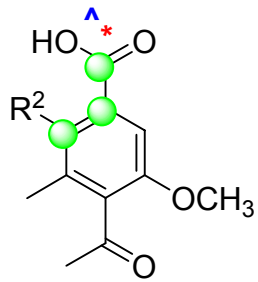
pyruvate

\+



5-acetyl-4-methoxy-
6-methyl-2-pyrone



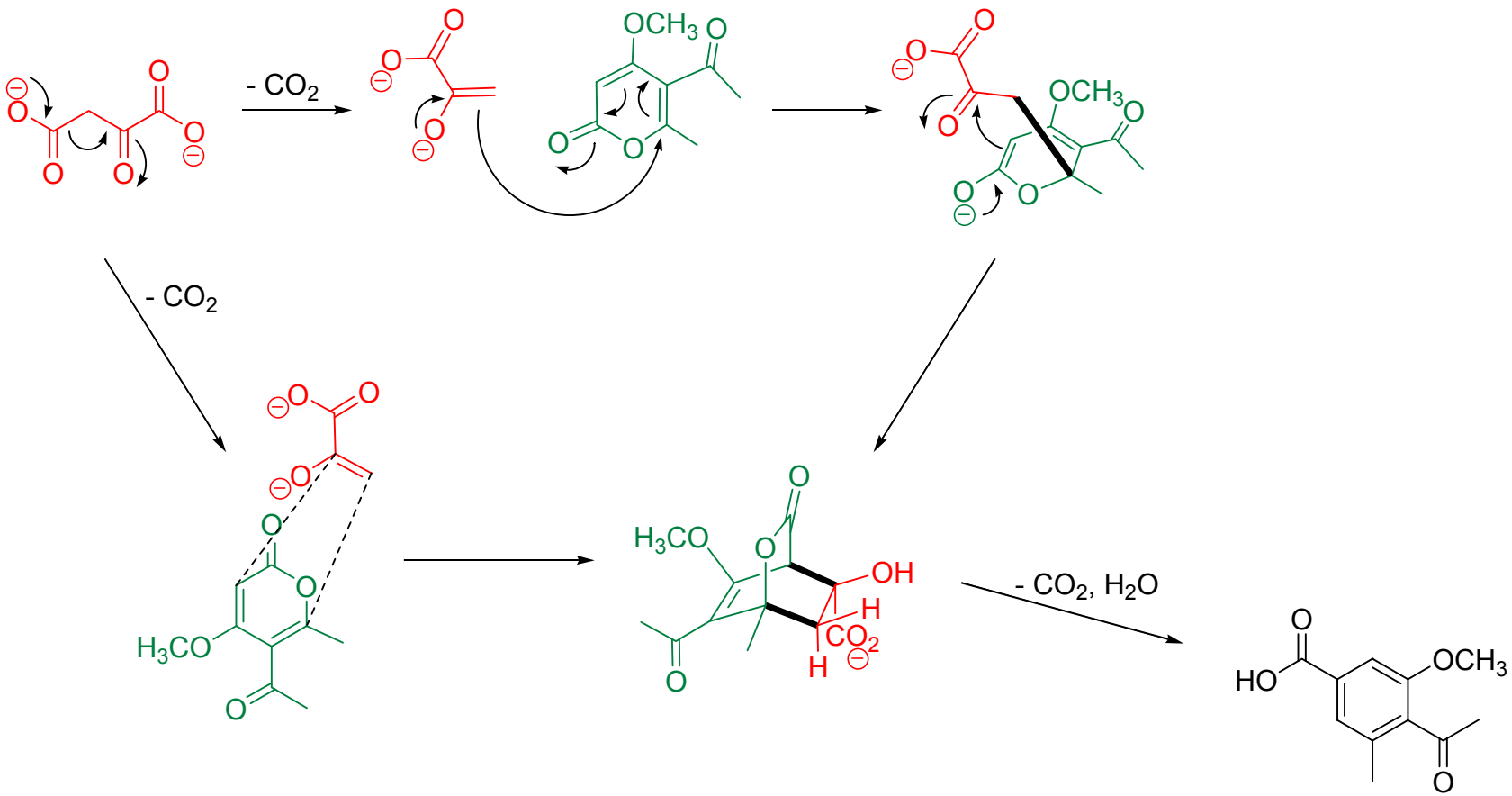


macrophomic acid

Oxaloacetate was determined to be the most likely three-carbon donor by isotope incorporation.

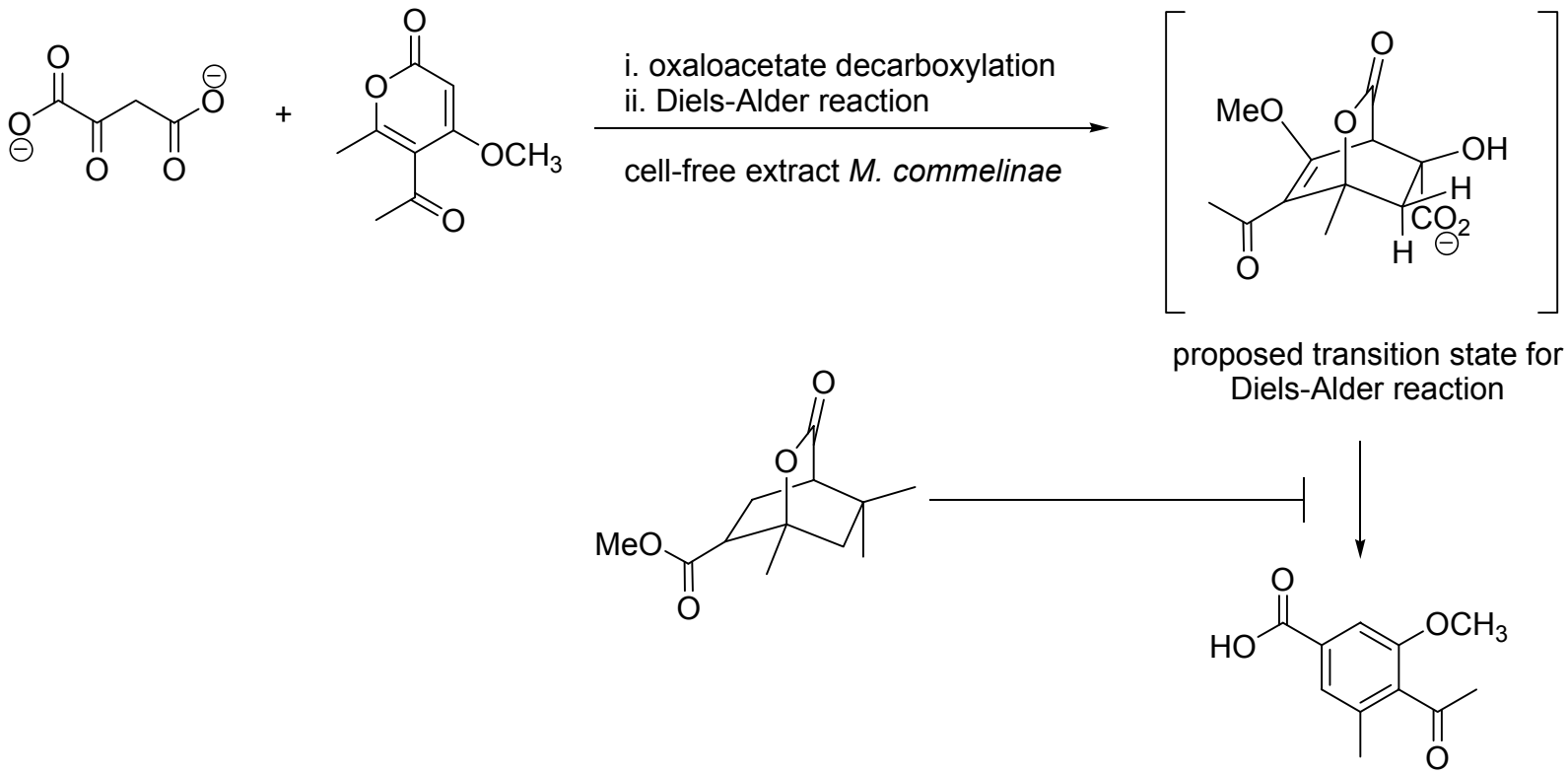
Oikawa, H.; Yagi, K.; Watanabe, K.; Honma, M.; Ichihara, A. *Chem. Commun.* **1997**, 97.

Michael/Aldol or Diels-Alder?



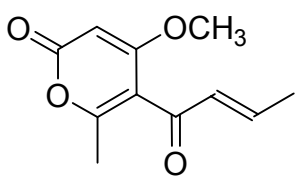
Watanabe, K.; Mie, T.; Ichihara, A.; Oikawa, H.; Honma, M. *J. Biol. Chem.* **2000**, *275*, 38393.

A Bicyclic Inhibitor of Macrophomate Synthesis



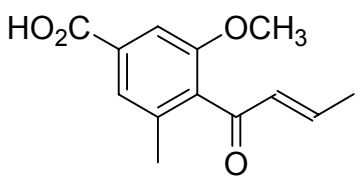
Oikawa, H.; Yagi, K.; Watanabe, K.; Honma, M.; Ichihara, A. *Chem. Commun.* **1997**, 97.

Biomimetic Synthesis of Pyrenochaetic Acid A

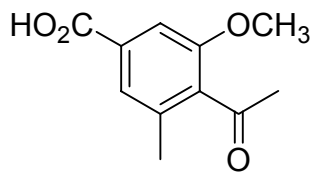


pyrenocine A

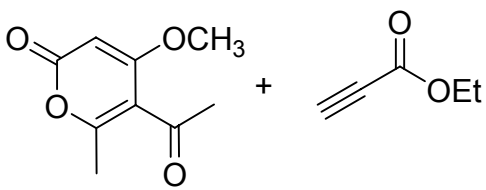
Pyrenochaeta terrestris
or
Macrophoma commelinae



pyrenochaetic acid A

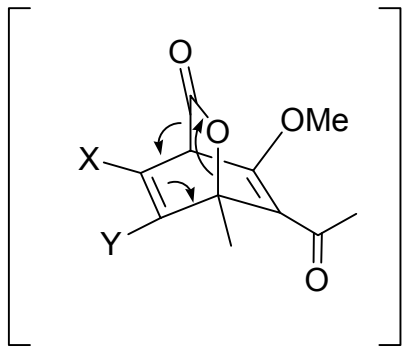


macrophomic acid

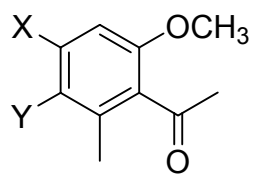


180-200 °C, sealed tube

neat, 17 h, 84%



$-CO_2$

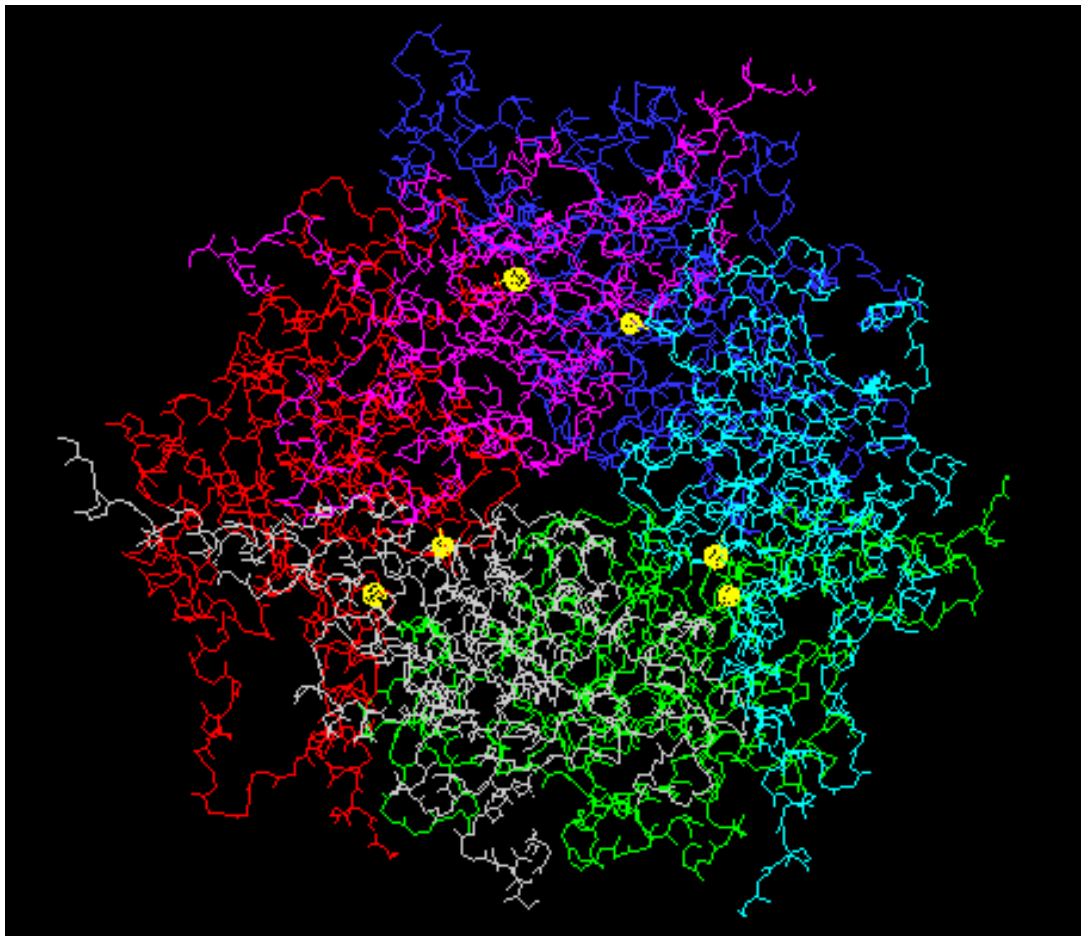


Product 1 closely resembles macrophomic acid.

product 1:
X = CO_2Et; Y = H
product 2:
X = H; Y = CO_2Et
1:2 ratio = 2:3

Sato, H.; Konoma, K.; Sakamura, S. *Agric. Biol. Chem.* **1979**, *43*, 2409.
Sato, H.; Konoma, K.; Sakamura, S. *Agric. Biol. Chem.* **1981**, *45*, 1675.
Ichihara, A.; Murakami, K.; Sakamura, S. *Tetrahedron* **1987**, *43*, 5245.

Crystal Structure of Macrophomate Synthase



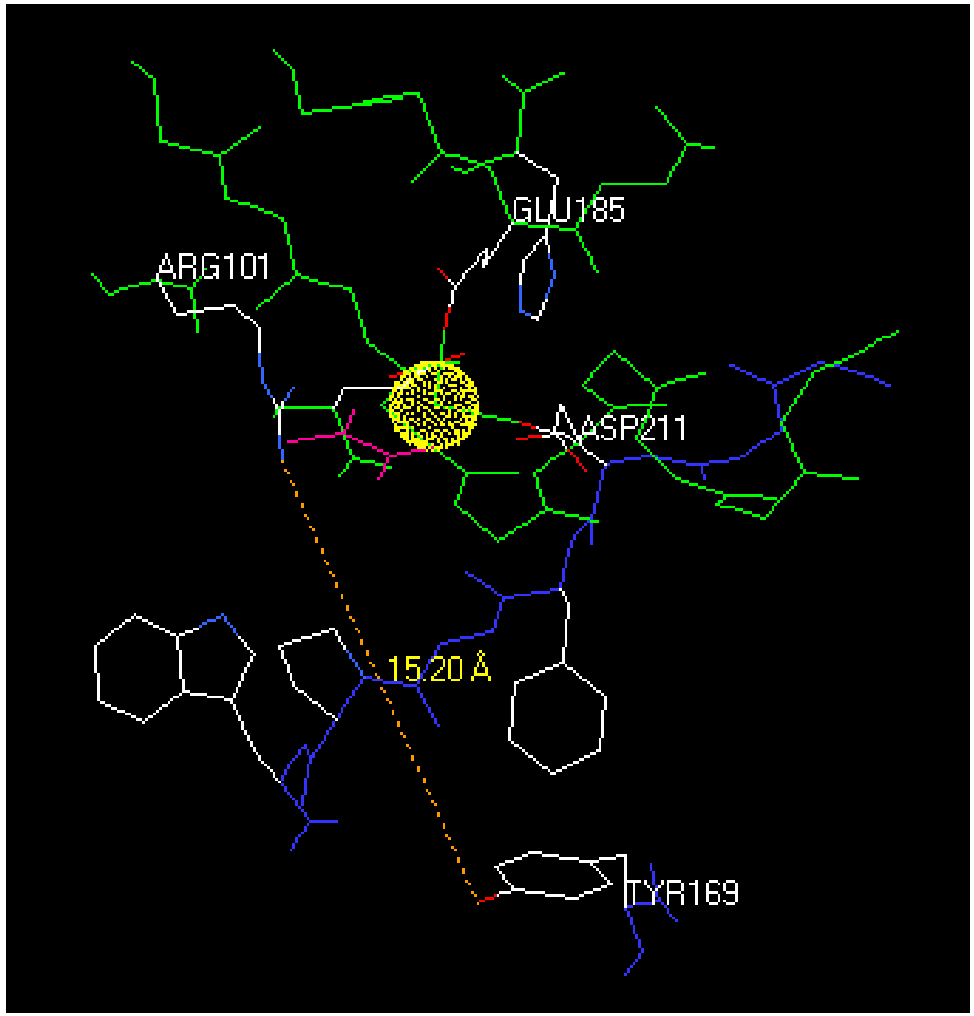
Structure 1.7 Å in complex with pyruvate and Mg^{2+}.

MW = 36 kDa.
Hexameric functional unit associated by hydrophobic interactions.

Each protomer is an 8-stranded β-barrel containing an octahedrally coordinated Mg^{2+} ion. Magnesium was known at the time to be necessary for oxaloacetate decarboxylation.

Ose, T.; Watanabe, K.; Mie, T.; Honma, M.; Watanabe, H.; Yao, M.; Oikawa, H.; Tanaka, I. *Nature* **2003**, *422*, 185.
Berman, H. M. et al. The Protein Data Bank. *Nucl. Acids Res.* **2000**, *28*, 235.
Image rendered with Deep View / Swiss PDB Viewer. http://www.expasy.org/spdbv/

Macrophomate Synthase Active Site



The Mg^{2+} ion stabilizes the pyruvate enolate.

Enzyme k_{cat}= 0.60±0.02 s^{-1}

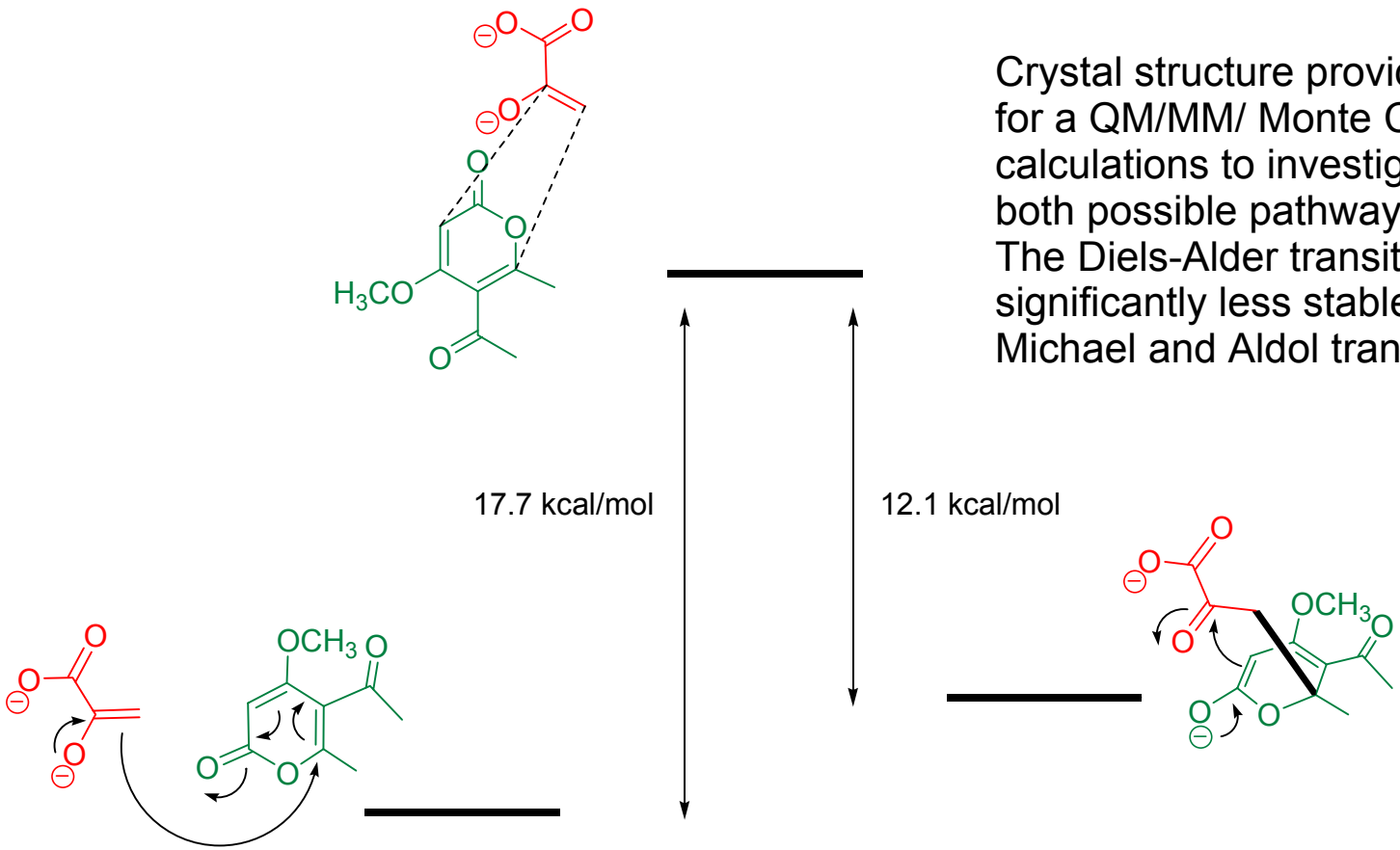
Arg101 and Tyr169 are thought to bind pyrone. Mutants lose MPS activity while retaining decarboxylase activity.

Steric congestion of peptide backbone allows access to one face of enolate.

Product inhibition is avoided by second decarboxylation and dehydration.

Ose, T., et al. *Nature* **2003**, *422*, 185.
Watanabe, K.; Oikawa, H.; Yagi, K.; Ohashi, S.; Mie, T.; Ichihara, A.; Honma, M. *J. Biochem.* **2000**, *127*, 467.
Berman, H. M. et al. The Protein Data Bank. *Nucl. Acids Res.* **2000**, *28*, 235.
Image rendered with Deep View / Swiss PDB Viewer. http://www.expasy.org/spdbv/

"A Bucket of Cold Water"



Crystal structure provided the basis for a QM/MM/ Monte Carlo calculations to investigate the energetics of both possible pathways.
The Diels-Alder transition state is significantly less stable than both Michael and Aldol transition states.

Guimarães, C. R. W.; Udier-Blagovic, M.; Jorgensen, W. L. *J. Am. Chem. Soc.* **2005**, *127*, 3577.
Wilson, E. *Chem. Eng. News.* **2005**, *83(18)*, 38.

Diels-Alderases: Summary

— Each of the three putative Diels-Alderases catalyzes one or several reactions prior to the cyclization step.
solanapyrone synthase: oxidation
lovastatin nonaketide synthase: polyketide chain formation
macrophomate synthase: decarboxylation

—General strategy appears to be entropy trapping of the substrate in the correct conformation to facilitate a [4+2] cycloaddition.

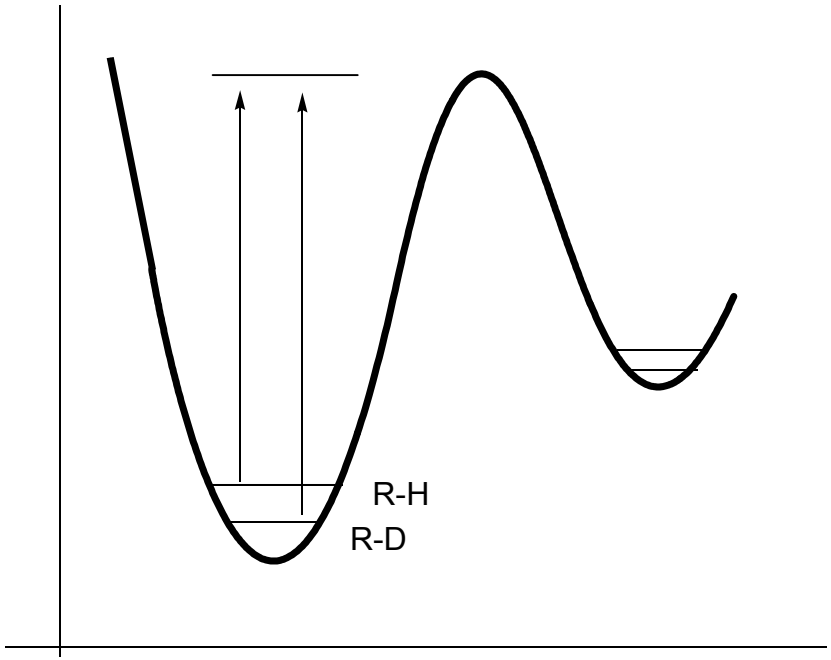
—"Proof that the proteins are accelerating the rates of the pericyclic Diels-Alder reaction remains to be rigorously established".
(RNA Diels-Alderases have shown up to 20,000 fold rate enhancement).

—"Calculations as well as work with mutants and inhibitors will have to clarify to what extent the Diels-Alder reaction in the enzyme active site of macrophomate synthase does indeed follow a concerted... pathway."

Pohnert, G. *ChemBioChem* **2003**, *4*, 713.
Stocking, E. M.; Williams, R. M. *Angew. Chem. Int. Ed.* **2003**, *42*, 3078.
Pitt, J. N.; Ferré-D'Amaré, A. R. *Nat. Struct. Mol. Biol.* **2005**, *12*, 206.

Kinetic Isotope Effect and Concerted Mechanisms

Kinetic Isotope Effect: difference in reaction rate when an atom is replaced by its isotope



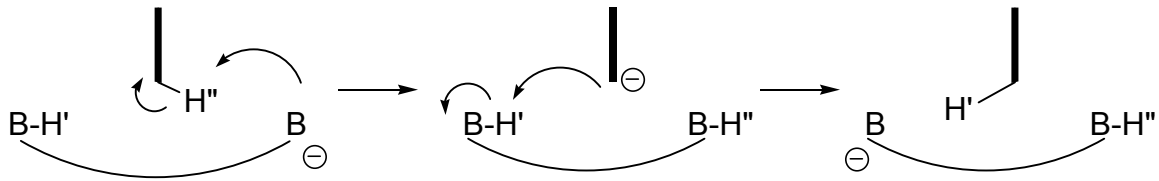
$E_n = h\nu\ (n + ½)$

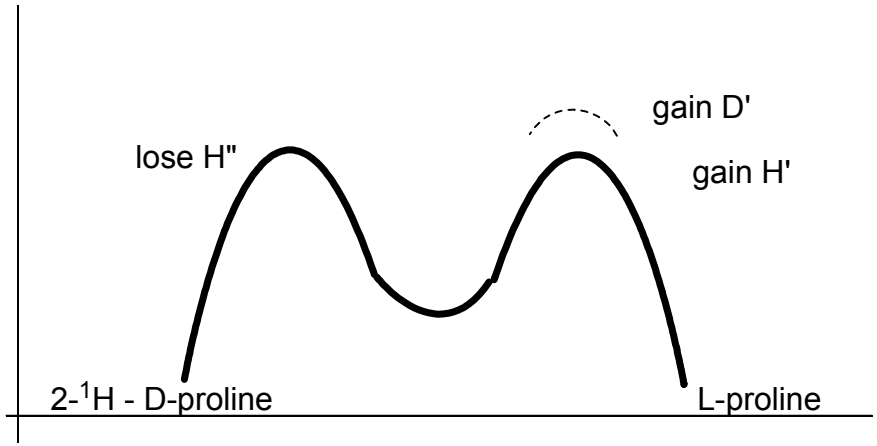
$\nu = 1/2\pi\ \mathrm{sqrt}(k/\mu)$

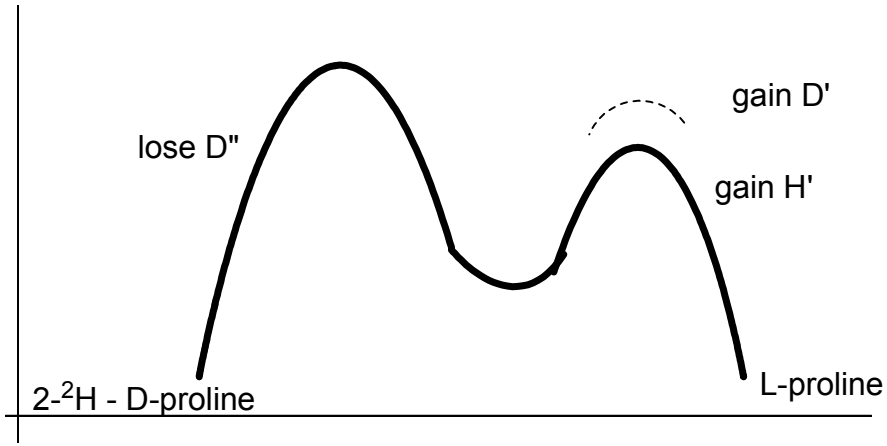
Carey, F. A.; Sundberg, R. J. in Advanced Organic Chemistry, 4th ed. **2000**, 222-225.

Kinetic Isotope Effect and Stepwise Mechanisms

Isotopic fractionation at the bond-making site measured as a function of the isotope at the bond-breaking site can be used to test for concertedness of enzymatic mechanism. Example: the enzyme proline racemase.







Belasco, J. G.; Albery, W. J.; Knowles, J. R. *J. Am. Chem. Soc.* **1983**, *105*, 2475.

Kinetic Isotope Effect and Concerted Mechanisms

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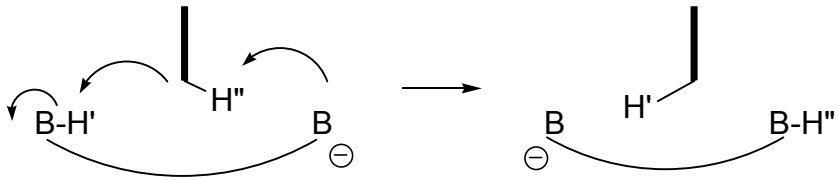
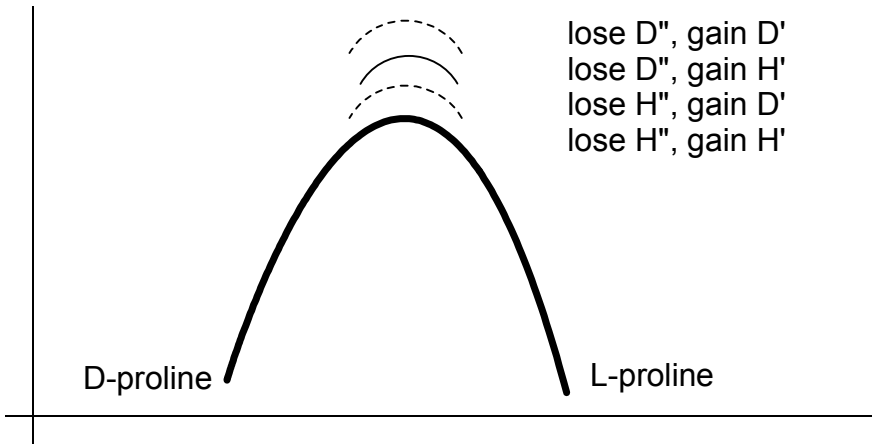




Belasco, J. G.; Albery, W. J.; Knowles, J. R. *J. Am. Chem. Soc.* **1983**, *105*, 2475.

Key Papers: Diels-Alder Reactions in Biology

Biosynthetic Diels-Alder Reactions (established and speculated)

— Stocking, E. M.; Williams, R. M. *Angew. Chem. Int. Ed.* **2003**, *42*, 3078.

— Oikawa, H.; Tokiwano, T. *Nat. Prod. Rep.* **2004**, *21*, 321.

— Oikawa, H. *Bull. Chem. Soc. Jpn.* **2005**, *78*, 537.

Antibody Diels-Alder Catalysis

— Hilvert, D.; Hill, K. W.; Nared, K. D.; Auditor, M. M. *J. Am. Chem. Soc.* **1989**, *111*, 9261.

— Romesberg, F. E.; Spiller, B.; Schultz, P. G.; Stevens, R. C. *Science* **1998**, *279*, 1929.

— Heine, A., et al. *Science* **1998**, *279*, 1934.

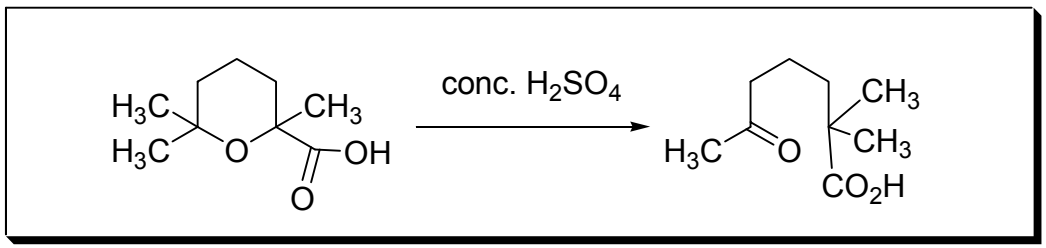
RNA Diels-Alder Catalysis

— Tarasow, T. M.; Tarasow, S. L.; Eaton, B. E. *Nature* **1997**, *389*, 54.

— Seelig, B.; Jäschke, A. *Chem. Biol.* **1999**, *6*, 167.

Problem: Mechanism?

Propose a mechanism for the acid catalyzed rearrangement of cinenic acid.



Meinwald, J.; Hwang, H. C.; Christman, D.; Wolf, A. P. *J. Am. Chem. Soc.* **1960**, *82*, 483.

Problem: Mechanism?

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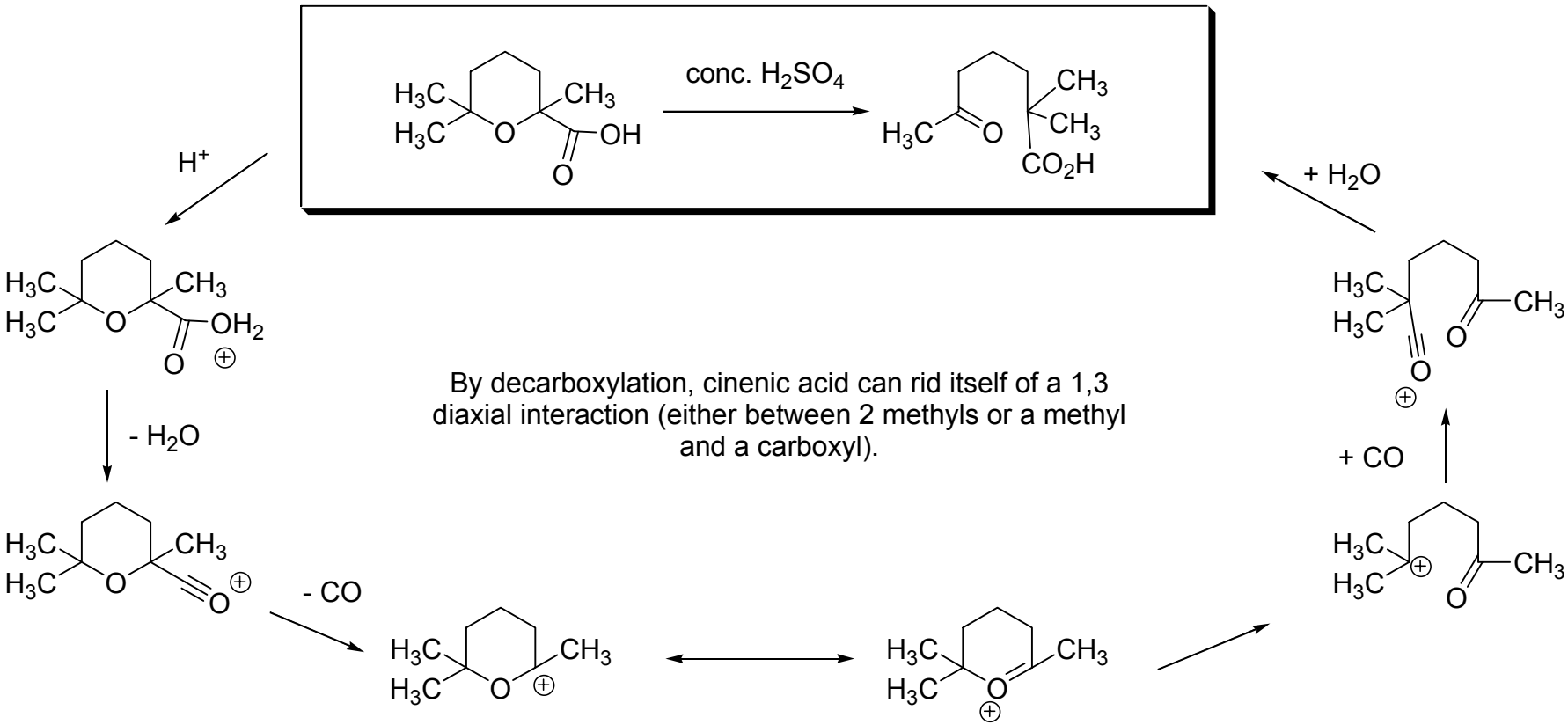

Meinwald, J.; Hwang, H. C.; Christman, D.; Wolf, A. P. *J. Am. Chem. Soc.* **1960**, *82*, 483.