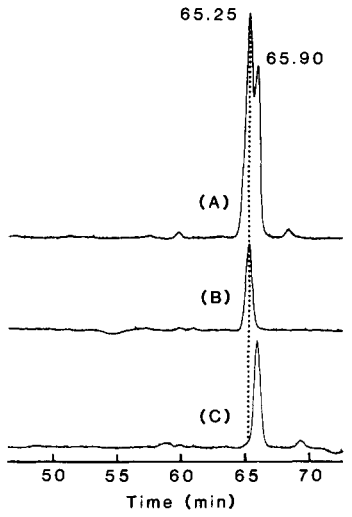
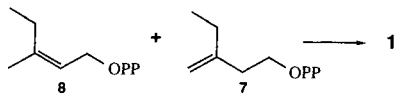


Figure 1. Gas chromatograms[8] of the products of biotransformation. (A) the product derived from **4a**, plus a synthetic mixture[9] of **5** and **6** (racemic); (B) the product derived from **4a**, plus natural 4-MeJH I (~50% of each); (C) the product derived from **4b**.

Scheme II



[5-^{3}H](4*R*)-4-methyl JH I were obtained. In order to compare these products with the natural 4-MeJH I, they were analyzed by capillary GLC[8] using conditions which separate the diastereomers from a chemically synthesized[9] mixture of racemic **5** and **6**. The separation was slight but sufficient to show that the material biotransformed from the *S* enantiomer **4a** generates only the faster eluting diastereomer, which comigrates with the natural product (Figure 1). The 4*R* isomer was slower eluting. Thus, 4-MeJH I was demonstrated to have the 4*S* configuration **5**, *opposite* to that of faranal.[2] These results also suggest that the epoxidation occurs on the enantiomer **4b** at the same face of the 10,11-double bond irrespective of the C-4 configuration. The configuration of the epoxy group is probably 10*R* as found in JH I and JH III,[10] but this requires further study.

Koyama et al.[11] have demonstrated that 3-ethyl-3-butenyl diphosphate (homoisopentenyl diphosphate, **7**) is converted by the action of isopentenyl diphosphate isomerase into not only (*Z*)-3-ethyl-2-butenyl diphosphate (**8**) but also (*E*)-3-methyl-3-pentenyl diphosphate (**2**). They have also shown that dihomogeranyl diphosphate (**1**) is formed as the intermediary product from **7** and **8** in the farnesyl diphosphate synthase reaction, giving ultimately trihomofarnesyl diphosphate,[12] the presumed precursor of JH 0 (Scheme II). Therefore, it is probable that the enzymatic and biological synthesis of 4-MeJH I reported herein represents the actual biosynthetic route. That is, isopentenyl diphosphate isomerase and farnesyl diphosphate synthase, both of which occur widely in organisms including insects,[13,14] are able to elaborate the 4-MeJH I carbon skeleton, given the availability of **7** presumably derived from homomevalonate.[15]

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(8) The GLC was carried out with a ~2-ng sample in isooctane with a 26-m Silar 10C glass capillary column (Applied Science). Conditions: splitless injection; initial temperature 80 °C for 0.5 min then programmed to 140 °C at 30 °C/min.

(9) The chemical synthesis will be published elsewhere.

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(12) Koyama, T.; Ogura, K.; Seto, S. *Chem. Lett.* **1973**, 401-404.

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(14) Koyama, T.; Matsubara, M.; Ogura, K. *J. Biochem.* (*Tokyo*) **1985**, *98*, 457-463.

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Mechanism-Based Inactivation of Catechol 2,3-Dioxygenase by 3-[(Methylthio)methyl]catechol

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The aromatic ring cleaving reactions catalyzed by the nonheme iron containing enzymes catechol 1,2-dioxygenase (pyrocatechase) and catechol 2,3-dioxygenase (metapyrocatechase)[1] are the prototypes of biological dioxygenations. All mechanisms proposed thus far for these reactions feature peroxidic species.[2] Organic peroxides efficiently oxidize thioethers to sulfoxides, so we have used thioether-containing substrates as probes for the presence of peroxidic intermediates in catalysis by dioxygenases.[3,4] In the course of these studies we made the unusual observation that 3-[(methylthio)methyl]catechol (**1**), a seemingly innocuous substrate analogue, was a mechanism-based inactivator of metapyrocatechase.

Compound **1**[5] was a good substrate for metapyrocatechase (k_{cat} = 2600 min^{-1} at 24 °C, K_m = 11 μM).[6] The only product detected, 2-hydroxy-6-oxo-7-(methylthio)-2,4-heptadienoic acid (**2**, Scheme I), resulted from cleavage of the aromatic ring between carbons 2 and 3. Compound **2** was characterized by its UV absorption spectrum (λ_{max} = 400 nm, typical of extradiol cleavage products[4]) and by conversion[6] to the picolinic acid derivative **3**.[7]

Preincubation of metapyrocatechase with **1** under aerobic conditions resulted in time-dependent irreversible inactivation of the enzyme (Figure 1).[8] The K_I for **1** was 6.6 μM, and extrapolation of the observed inactivation rates to infinite substrate concentration yielded k_{inact} = 0.12 min^{-1}. Thus the partition ratio[9] was 22 000 substrate turnovers per inactivation event. No inac-

(1) (a) Hayaishi, O.; Katagiri, M.; Rothberg, S. *J. Am. Chem. Soc.* **1955**, *77*, 5450-5451. (b) Nozaki, M.; Kagamiyama, H.; Hayaishi, O. *Biochem. Z.* **1963**, *338*, 582-590.

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(4) Pascal, R. A., Jr.; Huang, D.-S. *Arch. Biochem. Biophys.* **1986**, *248*, 130-137.

(5) The syntheses of compounds **1**, **4**, and **5** are outlined in the supplementary material.

(6) The methods for the preparation and assay of metapyrocatechase (from *Pseudomonas putida* ATCC 23973) and the characterization of enzymatic reaction products are essentially as described in ref 4.

(7) For **3**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.99 (s, 3 H, SCH_3), 3.84 (s, 2 H, CH_2), 3.93 (s, 3 H, CO_2CH_3), 7.58 (dd, 1 H, J = 8, 1 Hz, Ar H), 7.77 (t, 1 H, J = 8 Hz, Ar H), 7.96 (dd, 1 H, J = 8, 1 Hz, Ar H); MS, *m/z* 197 (M^+, 5%), 182 (M - CH_3, 8), 166 (M - OCH_3, 42), 151 (M - CH_3 - OCH_3, 100).

(8) Substrate (inactivator) concentrations lower than 10 μM were not used due to volume constraints, and concentrations above 120 μM were not used because of the onset of reversible substrate inhibition at 130 μM.

(9) Walsh, C. *Tetrahedron* **1982**, *38*, 871-909.

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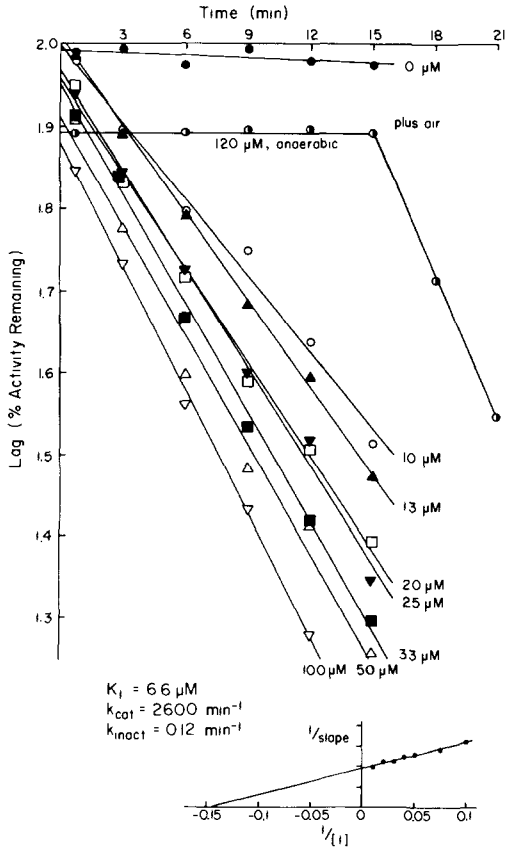
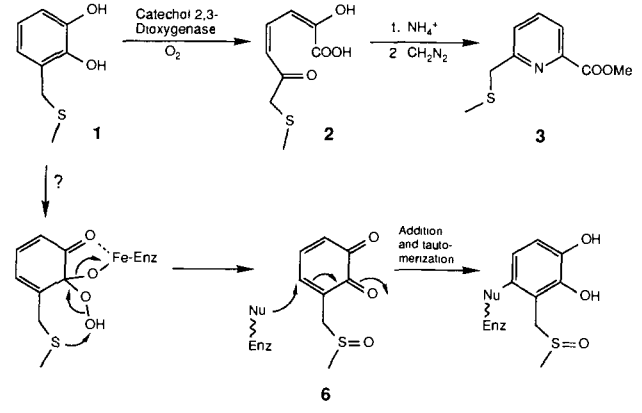


Figure 1. Time course of the inactivation of metapyrocatechase by 3-[(methylthio)methyl]catechol (**1**). In each inactivation experiment, metapyrocatechase (0.5 μg) and catalase (16 mg) were preincubated at 24 °C in potassium phosphate buffer (50 mM, pH 7.5, 100 mL) containing the indicated concentrations (0–100 μM) of **1**. At 3-min intervals, 15-mL aliquots were placed in 5-cm quartz cuvettes, and the enzyme activity was assayed by the changes in absorbance at 375 nm after the addition of catechol to 150 μM.

Scheme I



tivation was observed when enzyme was preincubated with **1** under anaerobic conditions (Figure 1), nor did inactivation occur in the absence of **1**. On the other hand, the presence of catalase did not protect the enzyme from inactivation, nor could inactive enzyme be reactivated by addition of iron salts or reducing agents in various combinations. Several experiments further characterized the inactivation process:

(1) A sample of **1** was exhaustively oxidized by metapyrocatechase, the protein was removed by ultrafiltration, and fresh metapyrocatechase was added to the filtrate. No inactivation was observed, ruling out the normal product **2** as the inactivating agent.

(2) 3-[(Methylthio)[^{3}H]methyl]catechol (**4**)[5] was prepared. Incubation of **4** with metapyrocatechase under aerobic conditions resulted in the incorporation of approximately 1.6 equiv of tritium into the inactivated enzyme.[10] This radiolabel was not removed by gel filtration or anion exchange chromatography, treatment with thiols, or dialysis of the protein against buffer containing sodium dodecyl sulfate, thus indicating that the enzyme is covalently modified in the course of the inactivation process.

(3) 3-Propylcatechol (**5**)[5] was prepared. Compounds **1** and **5** have essentially the same steric requirements, so any variance in the enzymic processing of the two substrates must result from electronic differences. Compound **5** is a good substrate for metapyrocatechase (k_{cat} = 4300 min^{-1}, K_m = 5 μM),[6] and like **1** it is cleaved between carbons 2 and 3,[12] but preincubation of the enzyme with **5** results in *no inactivation*, suggesting that the thioether moiety is essential for the inactivation process.

A plausible mechanism for the inactivation is illustrated in Scheme I. Sulfide oxidation by a peroxidic intermediate (or another oxygenating agent) would lead to the *o*-quinone **6**, a potent electrophile, which might be captured by an enzyme active site nucleophile to yield the inactive, covalently modified enzyme.

Acknowledgment. This work was supported by a grant from the National Institutes of Health (GM 31801).

Supplementary Material Available: Scheme for the synthesis of **1**, **4**, and **5** and spectrometric data for **1**, **4**, **5**, and **S1–S6** (2 pages). Ordering information is given on any current masthead page.

(10) Different preparations of metapyrocatechase, though homogeneous by SDS gel electrophoresis, vary in specific activity. The maximum activity observed thus far is 320 (μmol/min) mg^{-1} for enzyme containing 3.4 g-atoms of Fe/mol of enzyme.[11] The enzyme is a tetramer of identical subsunits, so the theoretical maximum activity is probably 380 (μmol/min) mg^{-1}. In two separate inactivation experiments, enzyme with specific activities of 14% and 39% of this maximum were labeled with 0.21 and 0.66 equiv of tritium, respectively.

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(12) The product 2-hydroxy-6-oxo-2,4-nonadienoic acid was characterized by conversion to methyl 6-propylpicolinate: ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.91 (t, 3 H, J = 7 Hz, $CH_2CH_2CH_3$), 1.69 (m, 2 H, $CH_2CH_2CH_3$), 2.81 (t, 2 H, J = 8 Hz, $CH_2CH_2CH_3$), 3.93 (s, 3 H, CO_2CH_3), 7.27 (dd, 1 H, J = 8, 1 Hz, Ar H), 7.67 (t, 1 H, J = 8 Hz, Ar H), 7.89 (dd, 1 H, J = 8, 1 Hz, Ar H).

Pentacyclo[4.3.1.0^{1,6}.0^{7,9}.0^{8,10}]decane: A Cyclopropane Edge-Bridged Prismane and Its Rearrangement to a Fulvene

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Short-bridged cyclophanes continue to be of theoretical and experimental interest.[1] Not surprisingly, the strain of these compounds is reflected in their historical sequence of appearance. This is illustrated by the first syntheses of [6]metacyclophane (**1a**; 1972[2]), [6]paracyclophane (**2a**; 1974[3]), [5]metacyclophane (**1b**; 1977[4]), and [5]paracyclophane (**2b**; 1985[5]). An extrapolation

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