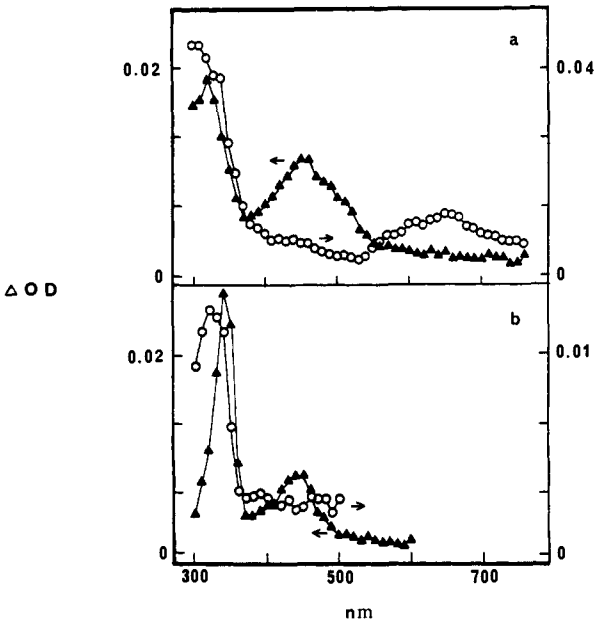

Figure 1. (a) Difference absorption spectrum of deaerated 10^{-3} M **1** in methanol 2.6 μs after 266-nm excitation alone (○) and in the presence of 0.2 M DABCO (▲). (b) Difference absorption spectrum of deaerated 2 mM **1** in 2% (vol) ethanolic 2-propanol 2 μs after a pulse radiolysis dose of ~2 krad (○) and difference absorption spectrum obtained 2.6 μs after 266-nm excitation of $\sim 10^{-4}$ M DCNB in methanol containing 0.2 M DABCO (▲).

been unsuccessful in promoting electron-transfer reaction of the tosylates. The reason for the failure to sensitize the tosylate reaction may lie in the thermodynamic and steric effects of electron transfer to carbohydrate tosylates. Events on a shorter time scale will be examined to address these questions.

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Synthesis and Absolute Configuration of 4-Methyl Juvenile Hormone I (4-MeJH I) by a Biogenetic Approach: A Combination of Enzymatic Synthesis and Biotransformation

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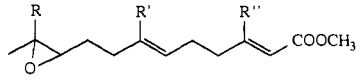
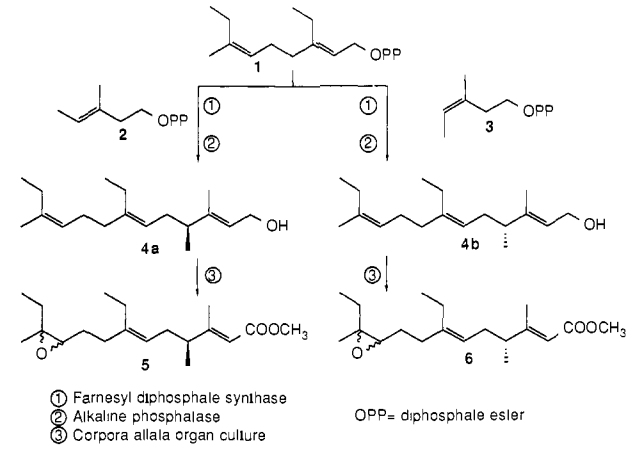
4-Methyl juvenile hormone I (4-MeJH I) (**5**) is a juvenile hormone (JH) isolated along with JH O from embryos of the tobacco hornworm, *Manduca sexta*. Bergot et al.[1] assigned its structure as a 4-methyl homologue of JH I, the first JH discovered.



R=R'=R''= Me, (10R)-JH III
R=Et, R'=R''=Me, JH II
R=R'=Et, R''=Me, (10R,11S)-JH I
R=R'=R''=Et, JH O

(1) Bergot, B. J.; Baker, F. C.; Cerf, D. C.; Jamieson, G.; Schooley, D. A. In *Juvenile Hormone Biochemistry*; Pratt, G. E., Brooks, G. T., Eds.; Elsevier: Amsterdam, 1981; pp 33-45.

Scheme I



The absolute configuration at C-4 is of particular interest as faranal,[2] the trail pheromone of the Pharaoh's ant, is a structural analogue of 4-MeJH I. We report the elucidation of the absolute configuration at C-4 of this compound by a biosynthetic approach.

The structure of 4-MeJH I led us to the following strategy for its (bio)synthesis: The farnesyl diphosphate synthase method,[2,3] which was successfully applied to the synthesis of faranal, seemed promising for the chiral synthesis of both (4*S*)-4-methyldihomofarnesol (**4a**) and (4*R*)-4-methyldihomofarnesol (**4b**), one of which should be the biosynthetic precursor of 4-MeJH I. [^{3}H]Farnesol is known[4] to be metabolized readily to [^{3}H]JH III by cultured corpora allata (the insect organ responsible for JH biosynthesis). If **4a** and **4b** are administered to corpora allata, one of them should be metabolized to a substance identical with natural 4-MeJH I.

First, tritium-labeled **4a** and **4b** were synthesized. The incubation mixture for the synthesis of **4a** contained, in a final volume of 400 mL, 8 mmol of TES [*N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid] buffer, pH 7.6, 2 mmol of $MgCl_2$, 4 mmol of 1,4-dithiothreitol, 50 mg of farnesyl diphosphate synthase purified about 50-fold from pig liver,[5] 35 μmol of (*E*)-3-methyl-3-pentenyl diphosphate (**2**),[3] and 25 μmol of [1-^{3}H]dihomogeranyl diphosphate (**1**). The latter was synthesized by phosphorylation of [1-^{3}H](2*E*,6*Z*)-3-ethyl-7-methyl-2,6-nonadien-1-ol (specific activity 1.3 Ci/mol) which had been prepared by reduction of the corresponding ester[6] with $LiAlT_4$. After incubation at 37 °C for 72 h, the mixture was treated with alkaline phosphatase to hydrolyze the diphosphate ester. The resulting alcohol was extracted with pentane and purified by TLC and HPLC to give 810 μg (12.3% yield based on **1**) of the 4*S*-(−) alcohol **4a**.[7] Similarly, the enzymatic reaction starting with **1** and (*Z*)-3-methyl-3-pentenyl diphosphate[3] (**3**) gave 590 μg (8.9% yield based on **1**) of the 4*R*-(+) alcohol **4b** (Scheme I).

The ^{3}H-labeled **4a** or **4b** (~50 μM) was incubated in 8-10 batches with 10 pairs of corpora allata from adult, female *M. sexta* (0-48-h old) in 100 μL of Medium 199 (Gibco), containing Hanks' salts and 1% bovine serum albumin. After a 5-h incubation at 28 °C, products were extracted and purified by reversed-phase HPLC (C_8 column; 70% CH_3CN). Quantification of 4-MeJH I was based on the level of ^{3}H. Thus, 1-2 μg of [5-^{3}H](4*S*)- and

(2) Kobayashi, M.; Koyama, T.; Ogura, K.; Seto, S.; Ritter, F. J.; Brüggemann-Rotgans, I. E. M. *J. Am. Chem. Soc.* **1980**, *102*, 6602-6604.
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(7) MS, *m/z* 264 (M^+, $C_{18}H_{32}O$), 246 (M - 18), 233 (M - 31), 163 (M - 18 - 83), 83 (base peak, C_6H_{11}); NMR (CCl_4) δ 0.96 (t, 6 H), 0.99 (d, 3 H), 1.56 (s, 3 H), 1.66 (s, 3 H), 1.5-1.6 (m, 1 H), 1.8-2.2 (m, 10 H), 4.19 (m, 2 H), 5.06 (m, 2 H), 5.42 (t, 1 H).

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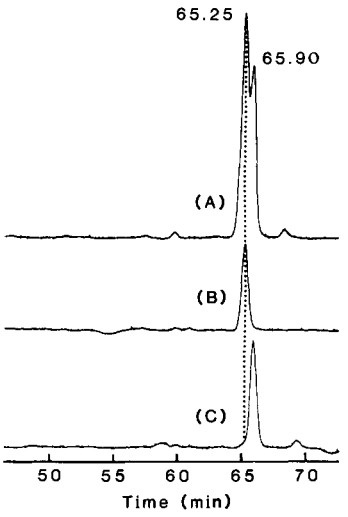
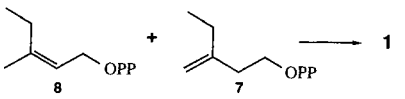


Figure 1. Gas chromatograms[8] of the products of biotransformation. (A) the product derived from **4a**, plus a synthetic mixture[9] of **5** and **6** (racemic); (B) the product derived from **4a**, plus natural 4-MeJH I (~50% of each); (C) the product derived from **4b**.

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.

(10) Judy, K. J.; Schooley, D. A.; Dunham, L. L., Hall, M. S., Bergot, B. J.; Siddall, J. B. *Proc. Natl. Acad. Sci.* U.S.A **1973**, *70*, 1509-1513.

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(15) Jennings, R. C.; Judy, K. J.; Schooley, D. A. *J. Chem. Soc., Chem. Commun.* **1975**, 21-22.

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-

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(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.

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