Biotransformation of α-Terpinene in Common Cutworm Larvae (*Spodoptera litura* Fabricius)

Mitsuo Miyazawa,* Takashi Wada, and Hiromu Kameoka

Department of Applied Chemistry, Faculty of Science and Engineering, Kinki University, Kowakae, Higashiosaka-shi, Osaka 577, Japan

α-Terpinene was mixed in an artificial diet at a concentration of 10 mg/g of diet, and the diet was fed to the last instar larvae of common cutworm, *Spodoptera litura* Fabricius. Metabolites were recovered from frass and analyzed spectroscopically. The α-terpinene was transformed mainly to 4-isopropyl-1,3-cyclohexadienoic acid and cumic acid. The allylic methyl group of α-terpinene was preferentially oxidized. The results indicate that the intestinal bacteria probably participated in the metabolism of α-terpinene. The aerobically active intestinal bacteria transformed α-terpinene to 4-isopropyl-1,3-cyclohexadienemethanol, and the anaerobically active intestinal bacteria transformed α-terpinene to *p*-cymene.

Keywords: *Common cutworm; Spodoptera litura Fabricius; larvae; biotransformation; α-terpinene; allylic oxidation; 4-isopropyl-1,3-cyclohexadienemethanol; 4-isopropyl-1,3-cyclohexadienoic acid; p-cymene; cumic alcohol; cumic acid; intestinal bacteria*

INTRODUCTION

Studies relating to the search for biologically active substances from natural products have been carried out extensively (Fujii and Yasuda, 1987; Fujii, 1989). Terpenoids constitute a large class of natural products that are isolated to be used as biologically active substances (allelochemicals). A great majority of these terpenoids are produced as plant secondary metabolites, and these terpenoids have been shown to have biological activity against microorganisms (Tahara et al., 1988), plants (Nishimura et al., 1982), and insects (Tahara et al., 1975).

Biotransformation is a useful way to produce biologically active terpenoids. We have investigated the biotransformation of terpenoids in mammals (Miyazawa et al., 1987, 1988, 1989) and by microorganisms (Miyazawa et al., 1990, 1991, 1992, 1993, 1994). In the present study, the biotransformation of terpenoids was attempted by the larvae of the common cutworm *Spodoptera litura* Fabricius. The reasons for using the larvae of *S. litura* as a biological catalyst are as follows: lepidopteran larvae feed on plants contained terpenoids as their diet and therefore possess a high level of enzymatic activity against terpenoids; the worm consumes a large amount of plants, making it possible to obtain more metabolites; and the worm is easy to rear on a laboratory scale. There are no reports in the literature on the biotransformation of terpenoids by lepidoptera, only reports about the biosynthesis of sex pheromone by beetles (Hughes, 1973; Renwick et al., 1975, 1976; Fish et al., 1979; Klimetzek and Francke, 1980; White et al., 1980; Byers, 1983; Francke and Vite, 1983). In the present paper, the biotransformation of α-terpinene (**1**) by the larvae of *S. litura* was investigated for the purpose of estimating possible metabolic pathways in insects. Compound **1** is a general terpenoid and is contained in various plants (*e.g*, *Citrus*, *Eucalyptus*, *Juniperus*, and *Pinus* species). This paper describes the metabolites from frass and the time course of metabolism. In addition, participation of intestinal bacteria in the metabolism of **1** is discussed.

* Author to whom correspondence should be addressed (telephone +81-6-721-2332; fax +81-6-727-4301).

MATERIALS AND METHODS

Chemicals. The α-terpinene (**1**) and *p*-cymene (**4**) were purchased from Tokyo Kasei Kogyo Company, Ltd. (Tokyo, Japan).

Gas Chromatography (GC). A Shimadzu GC-14A gas chromatograph equipped with a flame ionization detector, an OV-1 fused-silica capillary column (25-m length, 0.25-mm i.d.), and a split injection of 50:1 were used. Nitrogen at a flow rate of 1 mL/min was used as a carrier gas. The oven temperature was programmed from 80 to 240 °C at 4 °C/min. The injector and detector temperatures were 250 °C. The peak area was integrated with a Shimadzu C-R3A integrator.

Gas Chromatography−Mass Spectrometry (GC-MS). A Shimadzu GC-15A gas chromatograph equipped with a split injector was combined by direct coupling to a Shimadzu QP1000A mass spectrometer. The same type of column and the same temperature program as just described for GC were used. Helium at 1 mL/min was used as a carrier gas. The temperature of the ion source was 280 °C, and the electron energy was 70 eV. The electron-impact (EI) mode was used.

Infrared (IR) Spectroscopy. The IR spectra were obtained with a Perkin Elmer 1760X spectrometer. $CHCl_3$ was used as a solvent.

Nuclear Magnetic Resonance (NMR) Spectroscopy. The NMR spectra were obtained with a JEOL GSX-270 (270.05 MHz, ^1H, 67.80 MHz, ^{13}C) spectrometer.

Mass Rearing of Larvae. The larvae of *S. litura* were reared in plastic cases (200 × 300 mm wide, 100 mm high, 100 larvae/case) covered with a nylon mesh screen. The rearing conditions were as follows: 25 °C, 70% RH, and constant light. A commercial diet (Insecta LF; Nihon Nosan Kogyo Company, Ltd. Japan) was given to the larvae from the first instar. From the fourth instar, the diet was changed to an artificial diet composed of kidney beans (100 g), brewer's dried yeast (40 g), ascorbic acid (4 g), agar (12 g), and water (600 mL; Yushima et al., 1991).

Oral Administration of α-Terpinene (1). The artificial diet without the agar was mixed with a blender. Then, **1** was added directly into the blender at 10 mg/g diet. Agar was dissolved in water and boiled and then added into the blender. The diet was then mixed and cooled in a tray (220 × 310 mm wide, 30 mm high). The diet containing **1** was stored in a

S0021-8561(95)00296-2 CCC: $12.00

refrigerator until the time of administration. The last instar larvae (average weight, 0.5 g) were moved into new cases (100 larvae/case), and the diet was fed to the larvae in limited amounts. Groups of 500 larvae were fed for 7 days with 7.0 g of the diet containing **1**, then the artificial diet not containing **1** was fed to the larvae for an additional 3 days. The frass were collected daily (total 10 days) and stored in a solution of CH_2Cl_2 (500 mL).

Isolation and Identification of Metabolites from Frass. The frass were extracted three times with CH_2Cl_2 (500 mL each time). The extract solution was evaporated under reduced pressure, and the 6.1 g of the extract was obtained. The extract was distributed between 5% $NaHCO_3$ aq. and CH_2Cl_2, the CH_2Cl_2 phase was evaporated, and the neutral fraction (3.1 g) was obtained. The neutral fraction was analyzed by GC-MS, and metabolites **2** and **5** were identified by a comparison of established MS data. The alkali phase was acidified with 1 N HCl and distributed between water and CH_2Cl_2. The CH_2Cl_2 phase was evaporated, and the acidic fraction (2.5 g) was obtained. The acidic fraction was dissolved in CH_2Cl_2 (20 mL), and CH_2N_2 (5 mL) was added to the solution. The solution was evaporated, and the methylated fraction (2.8 g) was obtained. The methylated fraction was analyzed by GC-MS, and metabolites methylated **3** and methylated **6** were identified by a comparison of established MS data. The methylated fraction was subjected to silica-gel open-column chromatography (silica gel 60, 230−400 mesh, Merck) with a 9:1 *n*-hexane:$CHCl_3$ solvent system, and methylated **3** (1263 mg) was isolated. Methylated **3** was dissolved in 5% NaOH aq. (10 mL), and the solution was refluxed for 1 h at 100 °C. The solution was acidified with 1 N HCl and distributed between Et_2O and water. The Et_2O phase was evaporated yielding (1176 mg) of **3**. Metabolites **3** and methylated **3** were identified by a comparison of established MS, IR, and NMR data.

Injection of α-Terpinene (1). The last instar larvae (average weight, 0.5 g) were placed on ice (0 °C) to be put in a state of apparent death. Then, 1 mg (1.2 μL) of **1** (no solvent) was injected into the hemolymph with a microsyringe. The larvae were warmed back to 25 °C, and rearing was continued. The abdomen of larvae was cut with a scalpel, with 0.1 mL of hemolymph in the living body was collected. The concentration of metabolites in the hemolymph were determined 1, 3, 6, 12, and 24 h after injection of **1**. The hemolymph was acidified with 1 N HCl and distributed between Et_2O and a saturated solution of salt. The Et_2O phase was evaporated, and the extract was obtained.

Analysis of Metabolites in Living Body. The extracts of hemolymph were analyzed by GC, and metabolites **2** and **3** were identified by a comparison of retention times of identified metabolites. The concentration of metabolites was calculated from the peak area of GC spectra of the extracts of hemolymph, with **1** as an internal standard.

Injection of *p*-Cymene (4). Same procedure as described for **1**.

Incubation of Intestinal Bacteria with α-Terpinene (1). Petri dishes, pipets, and solutions were autoclaved. A GAM Broth (Nissui Pharmaceutical Company, Ltd., Tokyo, Japan) was adjusted to pH 9.0 and placed in Petri dishes at 10 mL/Petri dish. The fresh frass (5 g) of the last instar larvae were suspended in physiological saline (100 mL), and the suspension (1 mL) was pipetted into the medium. The medium without frass was also prepared for a blank experiment. These media were incubated (18 °C, darkness, 2 days) under aerobic and anaerobic conditions. After some growth of bacteria, **1** (10 mg/Petri dish) was added to the medium, and the incubation was continued. The percentage of metabolites in the medium were determined 12, 24, and 48 h after addition of **1**. The medium was acidified with 1 N HCl and distributed between Et_2O and a saturated solution of salt. The Et_2O phase was evaporated, and the extract was obtained. GC analysis, with **1** as an internal standard, was used for the quantitative analysis of metabolites. Under aerobic conditions, 8, 8, and 7 mg of metabolite were obtained at 12, 24, and 48 h, respectively; and under aerobic conditions, 9, 8, and 8 mg of metabolite were obtained at 12, 24, and 48 h, respectively.

Analysis of Metabolites in Culture Medium. The extracts of medium were analyzed by GC. Metabolite **2** was identified by comparison of its retention time with that of the identified metabolite, and metabolite **4** was identified by comparison of its retention time with that of the authentic sample. No bacteria grew on the control (no frass) medium. The percentage of metabolites was calculated from the peak areas in the GC spectra of the metabolites.

4-Isopropyl-1,3-cyclohexadienemethanol (2). Electron-impact MS (EIMS) *m/z* (rel. int.): 137 $[M\text{-}Me]^+$ (5), 127 (40), 119 (42), 109 (100), 81 (45), 43 (88).

4-Isopropyl-1,3-cyclohexadienoic Acid (3). EIMS *m/z* (rel. int.): 166 $[M]^+$ (33), 151 (14), 121 (60), 105 (56), 91 (34), 79 (100), 51 (42), 43 (61); IR (ν_{max} cm^{-1}): 3437, 2962, 1667, 1634, 1574, 1427, 1283, 1229; ^1H NMR ($CDCl_3$): δ 1.08 (6H, *d*, $J = 7.3$ Hz, Me-9 and Me-10), 2.22 (2H, *t*, $J = 12.4$ Hz, H-5), 2.40 (1H, *m*, $J = 7.3$ Hz, H-8), 2.45 (2H, *m*, $J = 12.4$ Hz, H-6), 5.86 (1H, *m*, $J = 5.4$ Hz, H-3), 7.15 (1H, *m*, $J = 5.4$ Hz, H-2); ^{13}C NMR ($CDCl_3$): δ 20.8 (*t*, C-9 and C-10), 21.4 (*q*, C-5), 25.5 (*q*, C-6), 35.2 (*d*, C-8), 116.5 (*d*, C-3), 124.0 (*d*, C-2), 136.9 (*s*, C-4), 155.9 (*s*, C-1), 172.8 (*s*, C-7).

4-Isopropyl-1,3-cyclohexadienoic Acid (3) as Methyl Ester. EIMS *m/z* (rel. int.): 180 $[M]^+$ (33), 137 (60), 121 (71), 105 (100), 91 (70), 77 (78), 59 (67), 51 (36), 43 (98); IR (ν_{max} cm^{-1}): 2963, 1708, 1583, 1436, 1268, 1226, 1091; ^1H NMR ($CDCl_3$): δ 1.07 (6H, *d*, $J = 6.5$ Hz, Me-9 and Me-10), 2.20 (2H, *t*, $J = 10.0$ Hz, H-5), 2.36 (1H, *m*, $J = 6.5$ Hz, H-8), 2.45 (2H, *m*, $J = 10.0$ Hz, H-6), 3.74 (3H, *s*, OMe), 5.82 (1H, *m*, $J = 5.9$ Hz, H-3), 7.01 (1H, *m*, $J = 5.9$ Hz, H-2); ^{13}C NMR ($CDCl_3$): δ 20.9 (*t*, C-9 and C-10), 21.9 (*q*, C-5), 25.5 (*q*, C-6), 34.2 (*d*, C-8), 51.9 (*t*, OMe), 116.4 (*d*, C-3), 124.7 (*d*, C-2), 134.6 (*s*, C-4), 154.4 (*s*, C-1), 168.0 (*s*, C-7).

Cumic Alcohol (5). EIMS *m/z* (rel. int.): 135 $[M\text{-}Me]^+$ (26), 43 (100).

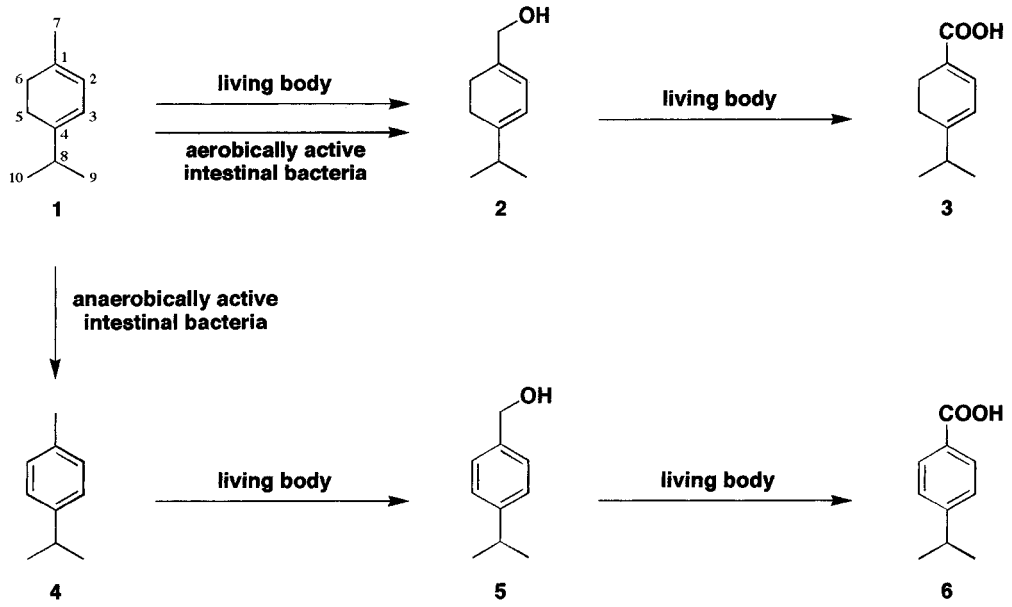
Cumic Acid (6) as Methyl Ester. EIMS *m/z* (rel. int.): 178 $[M]^+$ (34), 163 (100), 119 (69), 91 (63), 77 (41), 59 (44), 51 (39).

RESULTS AND DISCUSSION

Metabolites from Frass. α-Terpinene (**1**) was mixed in the diet of larvae at a high concentration to increase the production of potential metabolites. A concentration of **1** of 10 mg/g of diet was chosen as an upper limit for administration and apparently was not toxic to the larvae. Metabolites in frass were not only end metabolites but also intermediary metabolites at this concentration. The four metabolites isolated or detected from frass were identified as 4-isopropyl-1,3-cyclohexadienemethanol (**2**), 4-isopropyl-1,3-cyclohexadienoic acid (**3**), cumic alcohol (**5**), and cumic acid (**6**). The majority of metabolites were end metabolites **3** (71.7% in metabolites of **1**) and **6** (7.8%), and the remainder were intermediary metabolites **2** (3.8%) and **5** (0.5%). These metabolites were produced by oxidation at the 7-position of **1**, and allylic oxidation was the main metabolic pathway.

Time Course. The time course of the *in vivo* metabolism of **1** by the larvae was examined to better understand the metabolic pathways. A time course was determined by monitoring the concentration of metabolites in the hemolymph over time following the injection of a large dose of **1** into the larvae (the very large dose, 1 mg/larva, chosen did not affect the life of the larvae). Minor metabolites could not be detected by GC because various components contained in the extract from the hemolymph lowered the metabolite content in the extract. The hemolymph concentration of **1** and metabolites in the living body are shown in Figure 1. Starting material **1** disappeared completely from the hemolymph at 1 h after injection of **1**. Following the disappearance of **1**, intermediary metabolite **2** reached

Scheme 1. Metabolic Pathway of α-Terpinene (1) by the Larvae of *S. litura*



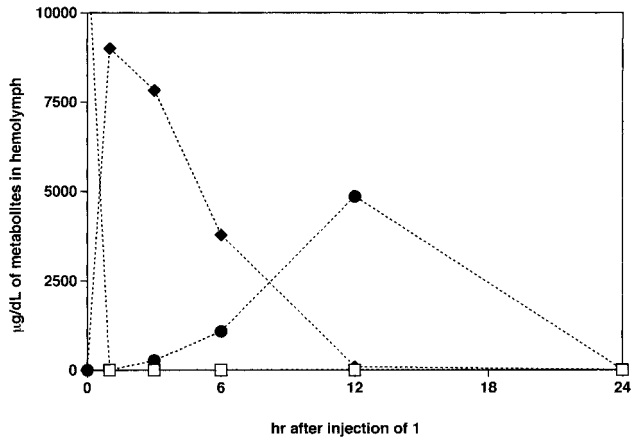


Figure 1. Hemolymph concentration of α-terpinene (**1**) and metabolites in the living body of *S. litura*. Concentration was calculated from the peak area in the GC spectra of the extracts of hemolymph, with **1** as an internal standard. Key: (□) α-terpinene (**1**); (◆) 4-isopropyl-1,3-cyclohexadienemethanol (**2**); (●) 4-isopropyl-1,3-cyclohexadienoic acid (**3**).

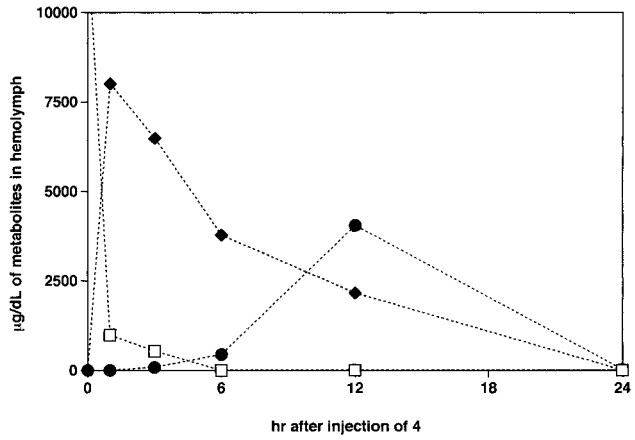


Figure 2. Hemolymph concentration of *p*-cymene (**4**) and metabolites in the living body of *S. litura*. Concentration was calculated from the peak area in the GC spectra of the extracts of hemolymph, with **4** as an internal standard. Key: (□) *p*-cymene (**4**); (◆) cumic alcohol (**5**); (●) cumic acid (**6**).

a maximum of 9000 μg/dL as soon as 1 h after injection of **1**, and then decreased slowly from 3 to 12 h. End metabolite **3** appeared in the hemolymph from 3 h after injection of **1**, and reached a maximum of 4860 μg/dL at 12 h. Metabolites **2** and **3** were almost eliminated from the larva at 24 h after injection of **1**.

In this experiment, minor metabolites **5** and **6** were not detected, as just described. Therefore, *p*-cymene (**4**) was used as a starting material to better understand the minor metabolic pathways. The hemolymph concentration of **4** and metabolites in the living body are shown in Figure 2. Starting material **4** almost disappeared from the hemolymph at 1 h after injection of **4**, and then decreased slowly from 3 to 6 h. Following the disappearance of **4**, intermediary metabolite **5** reached a maximum of 8010 μg/dL as soon as 1 h after injection of **4**, and then decreased slowly from 3 to 24 h. End metabolite **6** appeared in the hemolymph at 3 h after injection of **4**, and reached a maximum of 4050 μg/dL at 12 h. Metabolites **5** and **6** slightly remained in the hemolymph at 24 h after injection of **4**. These results indicate that carboxylic acids were produced by oxidation at the 7-position of **1** and **4**. However, the question of when the dehydrogenation proceeded was not answered. In addition, aldehydes were not detected as an intermediate from alcohols to acids during the metabolism of **1** and **4**; perhaps because labile aldehydes were oxidized immediately to acids.

Intestinal Bacteria. The *in vitro* metabolism of **1** by intestinal bacteria was also assessed. In this experiment, the bacteria contained in frass was regarded as intestinal bacteria. The time course of metabolism of **1** by the aerobically active intestinal bacteria is shown in Figure 3. Starting material **1** decreased slowly and reached 5% at 48 h after addition of **1**. Following a decrease of **1**, end metabolite **2** increased slowly and reached 95% at 48 h after addition of **1**.

The time course of metabolism of **1** by the anaerobically active intestinal bacteria is shown in Figure 4. Starting material **1** decreased slowly and reached 55% at 48 h after addition of **1**. Following a decrease of **1**, end metabolites **4** and **2** increased slowly and reached 35 and 10%, respectively, at 48 h after addition of **1**. Metabolites **2** and **4** were an intermediate on the metabolism of **1** by the larvae. It is interesting that the intestinal bacteria caused the same reaction as did the larvae after metabolism. However, it is unclear to what

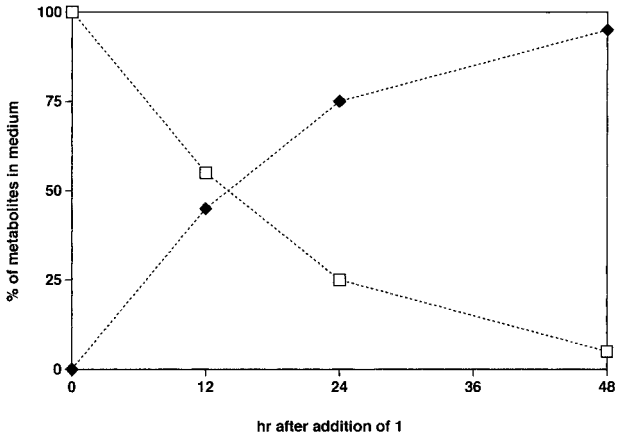


Figure 3. Time course of metabolism of α-terpinene (**1**) by aerobically active intestinal bacteria (10 mg of **1** was used as a starting material). The percentage was calculated from the peak area in the GC spectra of the metabolites (12 h, 8 mg; 24 h, 8 mg; 48 h, 7 mg); (□) α-terpinene (**1**); (◆) 4-isopropyl-1,3-cyclohexadienemethanol (**2**).

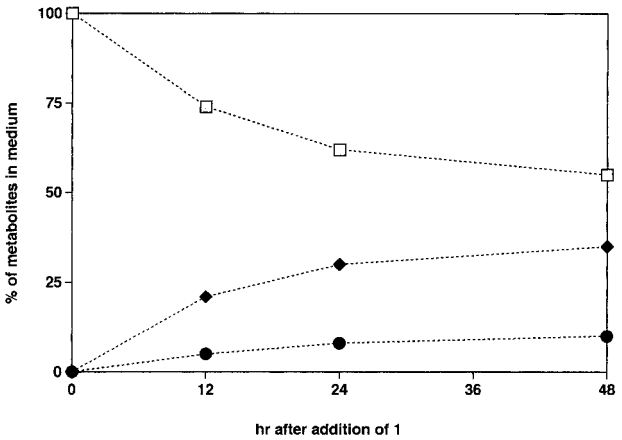


Figure 4. Time course of metabolism of α-terpinene (**1**) by anaerobically active intestinal bacteria (10 mg of **1** was used as a starting material). The percentage was calculated from the peak area in the GC of the metabolites (12 h, 9 mg; 24 h, 8 mg; 48 h, 8 mg). Key: (□) α-terpinene (**1**); (◆) *p*-cymene (**4**); (●) 4-isopropyl-1,3-cyclohexadienemethanol (**2**).

extent the intestinal bacteria participated in the metabolism of **1**.

In the present study of the biotransformation of **1**, the larvae produced carboxylic acids by oxidation at the 7-position of **1** (Scheme 1). It is difficult to compare the metabolism of **1** with other organisms because to date there are only two reports on the biotransformation of **1**; namely, a rabbit produced a glucuronide by oxidation at the 9-position of **1** (Asakawa et al., 1982); and a fungus, *Corynespora cassiicola*, produced a diol by oxidation at the 1,2-double bond of **1** (Abraham et al., 1986). There are, however, a few reports on the biotransformation of **4**. Mammals conjugated **4** mostly by oxidation at the 7- or 8- or 9-position (Ishida et al., 1981; Walde et al., 1983; Matsumoto et al., 1992). A brushtail possum produced a carboxylic acid by oxidation at the 7-position of **4** and degraded **4** to *p*-cresol (Southwell et al., 1980). Some species of microorganism degraded **4** by oxidation at the 7-position and 1,2-double bond (Madhyastha and Bhattacharyya, 1968; Wigmore and Ribbons, 1981; Ninnekar and Pujar, 1985; De Smet et al., 1989). Another species of microorganism produced carboxylic acids by oxidation at the 7- or 9-position of **4** (Yamada et al., 1965; Madhyastha and Bhattacharyya, 1968). These results indicate a difference in the oxidative position and the polarity of end metabolites between organisms (mammals, microorganisms, and insects). These differences are indications of the usefulness of this organism as a biological catalyst.

LITERATURE CITED

Abraham, W. R.; Stumpf, B.; Kieslich, K. Microbial transformation of terpenoids with 1-*p*-menthene skeleton. *Appl. Microbiol. Biotechnol.* **1986**, *24*, 24−30.

Asakawa, Y.; Ishida, T.; Toyota, M.; Takemoto, T. Presented at the 26th Symposium on the Chemistry of Terpens, Essential Oils and Aromatics of Japan, Yamagata, 1982; pp 153−155.

Byers, J. A. Bark beetle conversion of a plant compound to a sex specific inhibitor of pheromone attraction. *Science* **1983**, *220*, 624−626.

De Smet, M-J.; Friedman, M. B.; Gunsalus, I. C. Plasmid control of the *Pseudomonas aeruginosa* and *Pseudomonas putida* phenotypes and of linalool and *p*-cymene oxidation. *J. Bacteriol.* **1989**, *171*, 5155−5161.

Fish, R. H.; Browne, L. E.; Wood, D. L.; Hendry, L. B. Pheromone biosynthetic pathways: Conversion of deuterium labelled ispsdienol with sexual and enantioselectivity in *Ips paraconfusus* Lanier. *Tetrahedron Lett.* **1979**, 1465−1468.

Francke, W.; Vite, J. P. Oxygenated terpenes in pheromone systems of bark beetles. *Z. Angew. Entomol.* **1983**, *96*, 146−156.

Fujii, Y.; Yasuda, T. Biologically active substances in relation to plant interactions. *Bunseki* **1987**, *148*, 231−237.

Fujii Y. Prospective view of biological control by allelochemicals. *Nippon Dojo Hiryogaku Zasshi* **1989**, *60*, 240−245.

Hughes, P. R. *Dendroctonus*: Production of pheromones and related compounds in response to host monoterpenes. *Z. Angew. Entomol.* **1973**, *73*, 294−312.

Ishida, T.; Asakawa, Y.; Takemoto, T.; Aratani, T. Terpenoids biotransformation in mammals. III Biotransformation of α-pinene, *β*-pinene, pinane, 3-carene, carane, myrcene, and *p*-cymene in rabbits. *J. Pharm. Sci.* **1981**, *70*, 406−415.

Klimetzek, D.; Francke, W. Relationship between the enantiomeric composition of α-pinene in host trees and the production of verbenols in *Ips* species. *Experientia* **1980**, *36*, 1343−1344.

Madhyastha, K. M.; Bhattacharyya, P. K. Microbiological transformations of terpenes. XII Fermentation of *p*-cymene by a soil pseudomonad (PL-strain). *Indian J. Biochem.* **1968**, *5*, 102−111.

Matsumoto, T.; Ishida, T.; Yoshida, T.; Terao, H.; Takeda, Y.; Asakawa, Y. The enantioselective metabolism of *p*-cymene in rabbits. *Chem. Pharm. Bull.* **1992**, *40*, 1721−1726.

Miyazawa, M.; Kameoka, H. 10-Hydroxyfenchone: A new metabolite of (+)-fenchone in rabbits. *Chem. Express* **1987**, *2*, 547−550.

Miyazawa, M.; Kameoka, H. 9-Hydroxyfenchone: A new metabolite of (+)-fenchone in rabbits. *Chem. Express* **1988**, *3*, 231−234.

Miyazawa, M.; Kameoka, H.; Morinaga, K.; Negoro, K.; Mura, N. Hydroxycineole: Four new metabolites of 1,8-cineole in rabbits. *J. Agric. Food Chem.* **1989**, *37*, 222−226.

Miyazawa, M.; Yamamoto, K.; Noma, Y.; Kameoka, H. Bioconversion of (+)-fenchone to (+)-6-*endo*-hydroxyfenchone by *Aspergillus niger*. *Chem. Express* **1990a**, *5*, 237−240.

Miyazawa, M.; Yamamoto, K.; Noma, Y.; Kameoka, H. Bioconversion of (+)-fenchone to (+)-5-*endo*-hydroxyfenchone by *Aspergillus niger*. *Chem. Express* **1990b**, *5*, 407−410.

Miyazawa, M.; Funatsu, Y.; Kameoka, H. Biotransformation of (−)-α-bisabolol to (−)-α-bisabololoxid B by *Aspergillus niger*. *Chem. Express* **1990c**, *5*, 589−592.

Miyazawa, M.; Huruno, H.; Kameoka, H. Biotransformation of (+)-pulegone to (−)-1*R*-8-hydroxy-4-*p*-menthen-3-one by *Botrytis allii*. *Chem. Express* **1991a**, *6*, 479−482.

Miyazawa, M.; Nakaoka, H.; Hyakumachi, M.; Kameoka, H. Biotransformation of 1,8-cineole to (+)-2-*endo*-hydroxy-1,8-cineole by *Glomerella cingulata*. *Chem. Express* **1991b**, *6*, 667−670.

Miyazawa, M.; Noma, Y.; Yamamoto, K.; Kameoka, H. Biotransformation of 1,4-cineole to 2-*endo*-hydroxy-1,4-cineole by *Aspergillus niger*. *Chem. Express* **1991c**, *6*, 771−774.

Miyazawa, M.; Huruno, H.; Kameoka, H. Biotransformation of (+)-pulegone to piperitenone by *Botrytis allii*. *Chem. Express* **1991d**, *6*, 873−874.

Miyazawa, M.; Noma, Y.; Yamamoto, K.; Kameoka, H. Biotransformation of 1,4-cineole to 3-*endo*-hydroxy-1,4-cineole by *Aspergillus niger*. *Chem. Express* **1992a**, *7*, 125−128.

Miyazawa, M.; Funatsu, Y.; Kameoka, H. Biotransformation of (−)-α-bisabolol to (−)-α-tetrahydrobisabolen-2,5,6-triol by *Aspergillus niger*. *Chem. Express* **1992b**, *7*, 217−220.

Miyazawa, M.; Noma, Y.; Yamamoto, K.; Kameoka, H. Biohydroxylation of 1,4-cineole to 9-hydroxy-1,4-cineole by *Aspergillus niger*. *Chem. Express* **1992c**, *7*, 305−308.

Miyazawa, M.; Funatsu, Y.; Kameoka, H. Biotransformation of (−)-α-bisabolol to 6-methyl-2-(4′-methylcyclohexan-1′-yl)-2,5-epoxyheptane-3′,4′,6-triol by *Aspergillus niger*. *Chem. Express* **1992d**, *7*, 573−576.

Miyazawa, M.; Noma, Y.; Yamamoto, K.; Kameoka, H. Biohydroxylation of 1,4-cineole to 8-hydroxy-1,4-cineole by *Aspergillus niger*. *Chem. Express* **1992e**, *7*, 721−724.

Miyazawa, M.; Kakita, H.; Hyakumachi, M.; Kameoka, H. Enantioselective hydroxylation of (±)-piperitone to (−)-(4*R*, 6*S*)-6-hydroxy-piperitone by *Rhizoctonia solani*. *Chem. Express* **1993a**, *8*, 61−64.

Miyazawa, M.; Nankai, H.; Kameoka, H. Biotransformation of (−)-α-bisabolol to (2*S*, 5*S*, 1′*S*, 3′*R*, 4′*R*)-6-methyl-2-(4′-methylcyclohexane-1′-yl)-2,5-epoxyheptane-3′,4′,6-triol by *Glomerella cingulata*. *Chem. Express* **1993b**, *8*, 149−152.

Miyazawa, M.; Nankai, H.; Kameoka, H. Biotransformation of (−)-α-bisabolol to (2*S*, 5*S*, 1′*S*, 3′*S*, 4′*S*)-6-methyl-2-(4′-methylcyclohexane-1′-yl)-2,5-epoxyheptane-3′,4′,6-triol by *Glomerella cingulata*. *Chem. Express* **1993c**, *8*, 401−404.

Miyazawa, M.; Kakita, H.; Hyakumachi, M.; Kameoka, H. Preferential hydroxylation of (±)-piperitone to (−)-(4*S*, 6*S*)-6-hydroxypiperitone by *Rhizoctonia solani*. *Chem. Express* **1993d**, *8*, 569−572.

Miyazawa, M.; Nankai, H.; Kameoka, H. Microbial transformation of (+)-cedrol to 12-hydroxycedrol by *Glomerella cingulata*. *Chem. Express* **1993e**, *8*, 573−576.

Miyazawa, M.; Uemura, T.; Kameoka, H. Biotransformation of sesquiterpenoids, (−)-globulol and (+)-ledol by *Glomerella cingulata*. *Phytochemistry* **1994**, *37*, 1027−1030.

Ninnekar, H. Z.; Pujar, B. G. The metabolism of *p*-cymene by a Bacillus species. *Microbios Lett.* **1985**, *28*, 21−26.

Nishimura, H.; Nakamura, T.; Mizutani, J. Allelopathic substances, (±)-*p*-menthane-3,8-diols isolated from *Eucalyptus citriodora* Hook. *Agric. Biol. Chem.* **1982**, *46*, 319−320.

Renwick, J. A. A.; Hughes, P. R. Oxidation of unsaturated cyclic hydrocarbons by *Dendroctonus frontalis*. *Insect Biochem.* **1975**, *5*, 459−463.

Renwick, J. A. A.; Hughes, P. R.; Krull, I. S. Selective production of *cis*- and *trans*-verbenol from (−)- and (+)-α-pinene by a bark beetle. *Science* **1976**, *191*, 199−201.

Southwell, I. A.; Flynn, T. M.; Degabriele, R. Metabolism of α- and β-pinene, *p*-cymene, and 1,8-cineole in the brushtail possum, *Trichosurus* vulpecula. *Xenobiotica* **1980**, *10*, 17−23.

Tahara, S.; Yoshida, M.; Mizutani, J. Sex stimulant to the male American cockroach in *Conpositae* plants. *Agric. Biol. Chem.* **1975**, *39*, 1517−1518.

Tahara, S.; Hanawa, F.; Harada, Y.; Mizutani, J. A fungitoxin inducibly produced by dandelion leaves treated with cupric chloride. *Agric. Biol. Chem.* **1988**, *52*, 2947−2948.

Walde, A.; Ve, B.; Scheline, R. R. *p*-Cymene metabolism in rats and guinea pigs. *Xenobiotica* **1983**, *13*, 503−512.

White, R. A., Jr.; Agosin, R.; Franklin, R. T.; Webb, J. W. Bark beetle pheromones: Evidence for physiological synthesis mechanisms and the ecological implications. *Z. Angew. Entomol.* **1980**, *90*, 255−274.

Wigmore, G. J.; Ribbons, D. W. Selective enrichment of *Pseudomonas* spp. Defective in catabolism after exposure to halogenated substrates. *J. Bacteriol.* **1981**, *146*, 920−927.

Yamada, K.; Horiguchi, S.; Takahashi, J. Studies on the utilization of hydrocarbons by microorganisms part VI. Screening of aromatic hydrocarbon-assimilating microorganisms and cumic acid formation from *p*-cymene. *Agric. Biol. Chem.* **1965**, *29*, 943−948.

Yushima, K.; Kamano, S.; Tamaki, Y. *Rearing Methods of Insects*; Japan Plant Protection Association: Tokyo, Japan, 1991; pp 214−218.

Received for review May 16, 1995. Revised manuscript received March 28, 1996. Accepted June 7, 1996.[⊗]

JF9502966

[⊗] Abstract published in *Advance ACS Abstracts,* August 1, 1996.