Biotransformations of Acyclic Terpenoids, (±)-*trans*-Nerolidol and Geranylacetone, by *Glomerella cingulata*

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Microbial transformations of (±)-*trans*-nerolidol and geranylacetone were carried out with a plant pathogenic fungus, *Glomerella cingulata*. (±)-*trans*-Nerolidol and geranylacetone were hydrated at a remote double bond as the main metabolic pathway. A large amount of (*E*)-3,7,11-trimethyl-1,6-dodecadiene-3,11-diol and small amount of (*E*)-3,7,11-trimethyl-1,6-dodecadiene-3,10,11-triol were obtained from (±)-*trans*-nerolidol. Geranylacetone was transformed to (*E*)-10-hydroxy-6,10-dimethyl-5-undecen-2-one as the major metabolite. (*E*)-9,10-Dihydroxy-6,10-dimethyl-5-undecen-2-one, (*E*)-6,10-dimethyl-5,9-undecadien-2-ol, (*E*)-6,10-dimethyl-5-undecene-2,9,10-triol, and (*E*)-6,10-dimethyl-5-undecene-2,10-diol were also obtained from geranylacetone. The structures of metabolic products were determined by spectroscopic data.

Keywords: *Biotransformation; microbial transformation; Glomerella cingulata; plant pathogenic fungus; (±)-trans-nerolidol; geranylacetone*

INTRODUCTION

Phytopathogens are known for their abilities to transform natural compounds. For example, kievitone (phytoalexin) produced by *Phaseolus vulgaris* is transformed by *Fusarium solani* to less toxic compounds (Kuhn et al., 1977). Some metabolic products of microbial transformation have bioactivity. *Klebsiella oxytoca* and *Erwinia uredovora*, which are not phytopathogens but epiphytic bacteria [constituted of epiphytic microflora on yacon (*Polymnia sonchifolia*) leaves], converted hydroxycinnamic acids into hydroxystyrenes decarboxylatively. This decarboxylation was for the bacteria a detoxification of hydroxycinnamic acids of plants, but the metabolites were toxic to plant pathogenic fungus (Hashidoko et al., 1993). So, as part of our continuing program to search for and produce bioactive compounds from natural products, we screened some plant phathogens to transform terpenoids, and we found *Glomerella cingulata* has the ability to transform terpenoids. *G. cingulata* is widely distributed in the world, infecting various plants and causing anthracnose. However, there has been little report of the biotransformation of organic compounds using *G. cingulata* (Kieslich et al., 1980; Tsuda, 1987); therefore, we investigated the microbial transformation of cyclic terpenoids using *G. cingulata* (Miyazawa et al., 1991, 1994, 1995a,b,d,e). Then we tried the microbial transformation of acyclic terpenoids using *G. cingulata*. Acyclic terpenoids are widely distributed in nature, and they are important biosynthetic precursors of cyclic terpenoids in living organisms such as higher plants. If it is possible to produce new cyclic terpenoids or synthetic precursors of bioactive compounds from acyclic terpenoids by microbial transformation, it will be valuable for synthetic organic chemistry. Therefore, we recently reported the biotransformation of acyclic sesquiterpenoids (±)-*cis*-nerolidol (**10**) and nerylacetone (**12**) by *G. cingulata* (Miyazawa et al., 1995c). Compounds **10** and **12** were mainly oxidized at the remote double bond to *vic*-diol (Figure 1). In the present paper, to investigate the influence of *cis*/*trans* isomerism on the biotransformation, microbial transformations of (±)-*trans*-nerolidol (**1**) (isomer of **10**) and geranylacetone (**4**) (isomer of **12**) using *G. cingulata* were investigated. Unlike the biotransformation of **10** and **12** (*cis*-form), **1** and **4** (*trans*-form) were mainly hydrated at the remote double bond by *G. cingulata*. **1** was transformed to (*E*)-3,7,-11-trimethyl-1,6-dodecadiene-3,11-diol (**2**) and (*E*)-3,7,-11-trimethyl-1,6-dodecadiene-3,10,11-triol (**3**). **4** was transformed to (*E*)-10-hydroxy-6,10-dimethyl-5-undecen-2-one (**7**) as the major metabolite. (*E*)-9,10-Dihydroxy-6,10-dimethyl-5-undecen-2-one (**5**), (*E*)-6,10-dimethyl-5,9-undecadien-2-ol (**6**), (*E*)-6,10-dimethyl-5-undecene-2,9,10-triol (**8**), and (*E*)-6,10-dimethyl-5-undecene-2,10-diol (**9**) were also formed.

There are some reports of microbial transformation of **1** and **4** by other microorganisms. **1** was transformed to **3**, (6*E*,10*E*)-3,7,11-trimethyl-1,6,10-dodecatriene-3,-12-diol, and other metabolites (Abraham et al., 1985, 1989, 1990; Abraham and Stumpf, 1987; Alfmann et al., 1988; Holmes et al., 1990; Madyastha and Gurura, 1993). **4** was transformed to **5**, **6**, and other metabolites (Abraham et al., 1989; Abraham and Stumpf, 1987; Juttner and Hans, 1986; Madyastha and Gurura, 1993). However, **2** and **7**−**9** had not been obtained by other microbial transformations. The present paper is the first report of biotransformation of **1** to **2** and of **4** to **7**−**9**.

EXPERIMENTAL PROCEDURES

General Procedure. Thin-layer chromatography (TLC) was performed on precoated plates (silica gel 60 F_{254}, 0.25 mm, Merck). Solvent systems were hexane/ethyl acetate [1:1 (v/v) solvent system 1 (S1); and 1:4 (v/v) solvent system 2 (S2)]. Spots were visualized by spraying 1% vanillin in 96% sulfuric acid followed by brief heating. Gas chromatography (GC) was performed on a Shimadzu GC-2D gas chromatograph equipped with a flame ionization detector (FID). The column used was a fused silica capillary column (OV-1, 30 m × 0.25 mm i.d.). Chromatographic conditions were as follows: column temperature, raised from 140 to 260 °C at 4 °C min^{-1}; injector and

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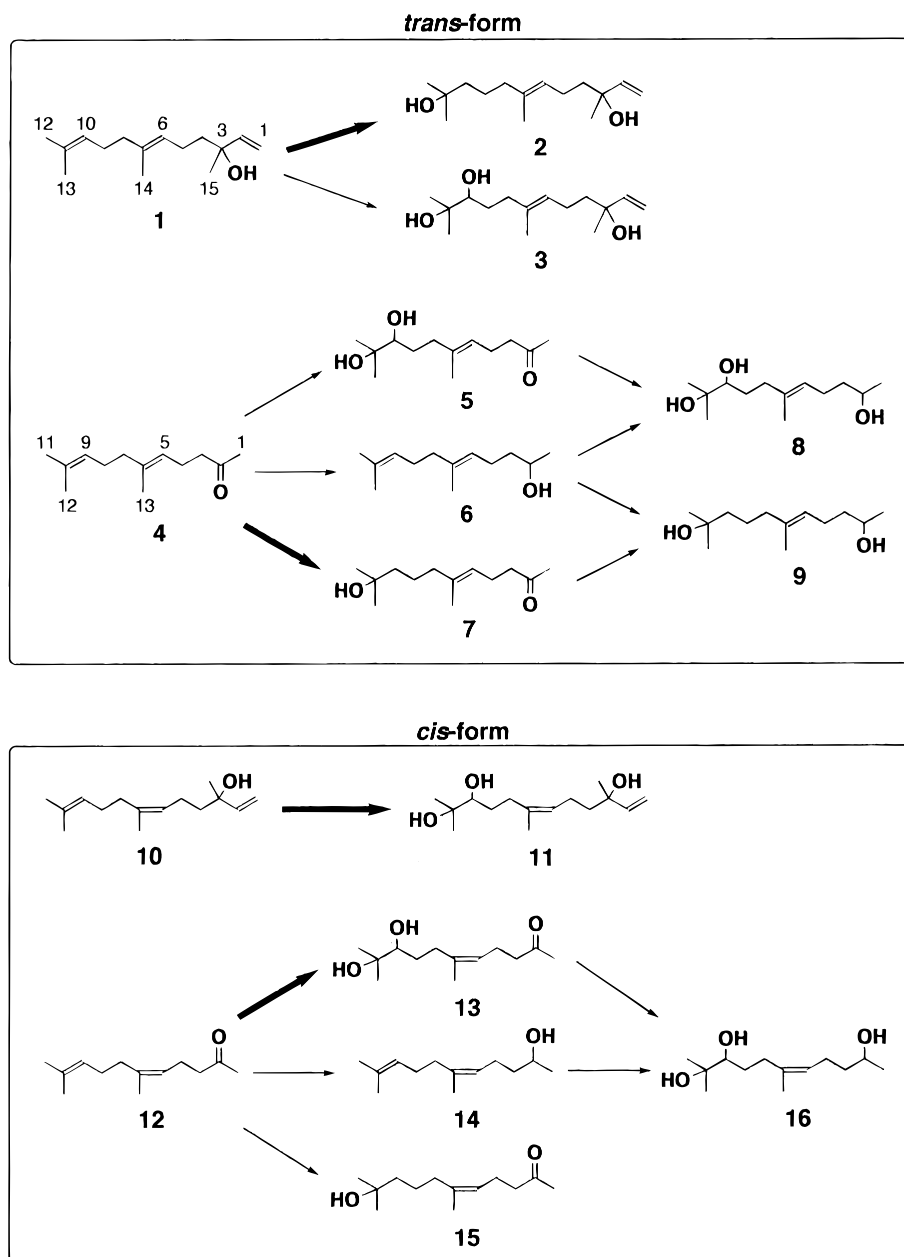

Figure 1. Structures of substrates and metabolic products.

detector temperature, 270 °C; carrier gas, N_2 at 1 mL min^{-1}. Yields of individual constituents were determined by response factor for peak areas as measured by a Shimadzu chromatopac C-R3A. Electron impact mass spectra (EI-MS) measurements were obtained using gas chromatography−mass spectrometry (GC−MS). GC−MS was performed on a Shimadzu QP1000A mass spectrometer interfaced with a Shimadzu GC-15A gas chromatograph fitted with a column (OV-1, 30 m × 0.25 mm i.d.). Chromatographic conditions were as follows: column temperature, raised from 140 to 260 °C at 4 °C min^{-1}; injector and detector temperature, 270 °C; carrier gas, He at 1 mL min^{-1}. Fast atom bonberedment mass spectra (FAB-MS) were obtained on a JEOL JMS-HX 100 mass spectrometer, and the matrix was 3-nitrobenzyl alcohol. Infrared (IR) spectra were determined with a Perkin-Elmer 1760-x IR Fourier transform spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL FX 200 NMR spectrometer (^1H NMR, 270.05 MHz; ^{13}C NMR, 67.80 MHz) and JEOL GX 500 NMR spectrometer (^1H NMR, 500.00 MHz; ^{13}C NMR, 125.65 MHz). Tetramethylsilane (TMS) was used as the internal reference (δ 0.00) for ^1H-NMR spectra measured in $CDCl_3$. Residual $CHCl_3$ was used as internal reference (δ 77.00) for ^{13}C-NMR spectra measured in $CDCl_3$.

Preculture of *G. cingulata*. Spores of *G. cingulata* (the strain isolated from diseased grape was a gift from Dr. Hyakumachi, Gifu University), which have been preserved on potato dextrose agar (PDA) at 4 °C, were inoculated into 200 mL of sterilized culture medium (1.5% saccharose, 1.5% glucose, 0.5% polypepton, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl, 0.1% K_2HPO_4, and 0.001% $FeSO_4 \cdot 7H_2O$ in distilled water) in a 500 mL shaking flask, and the flask was shaken (reciprocating shaker, 100 rpm) at 27 °C for 3 days.

Time Course Experiment. Precultured *G. cingulata* (1 mL) was transferred into two 100 mL Erlenmeyer flasks containing 50 mL of medium and was stirred (ca. 100 rpm) for 3 days. After the growth of *G. cingulata*, **1** (100 mg) and **4** (100 mg) were added into the medium, respectively, and cultivated 10 (for **1**) or 8 (for **4**) more days. Every other day 5 mL of the culture medium was removed, acidified to pH 2 with 1 M HCl, and saturated with NaCl. Then, the culture medium was extracted with diethyl ether. The extract was analyzed by GC and TLC. The ratio between the substrate and metabolic products was determined on the basis of the peak area of GC and is shown in Figures 2 and 3.

Biotransformation of *trans*-Nerolidol (1) for 10 Days. Precultured *G. cingulata* (5 mL) was transferred into two 1 L stirred fermentors containing 500 mL of medium. Cultivation was carried out at 27 °C with stirring (ca. 100 rpm) for 3 days under aeration (200 mL min^{-1}). After the growth of *G.*

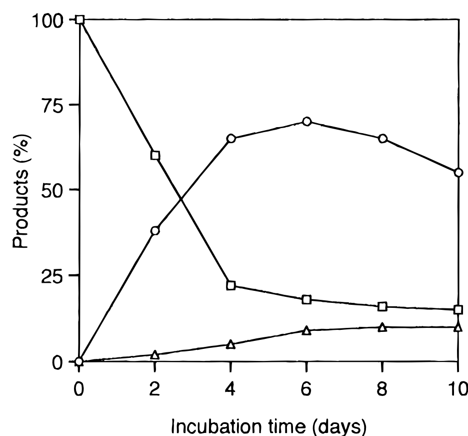

Figure 2. Time course in the biotransformation of **1** by *G. cingulata*: (□) (±)-*trans*-nerolidol (**1**); (○) (*E*)-3,7,11-trimethyl-1,6-dodecadiene-3,11-diol (**2**); (△) (*E*)-3,7,11-trimethyl-1,6-dodecadiene-3,10,11-triol (**3**).

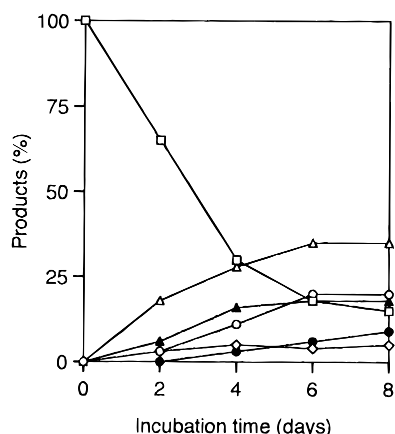

Figure 3. Time course in the biotransformation of **4** by *G. cingulata*: (□) geranylacetone (**4**); (○) (*E*)-9,10-dihydroxy-6,-10-dimethyl-5-undecen-2-one (**5**); (◇) (*E*)-6,10-dimethyl-5,9-undecadien-2-ol (**6**); (△) (*E*)-10-hydroxy-6,10-dimethyl-5-undecen-2-one (**7**); (●) (*E*)-6,10-dimethyl-5-undecene-2,9,10-triol (**8**); (▲) (*E*)-6,10-dimethyl-5-undecene-2,10-diol (**9**).

cingulata, **1** (1.00 and 1.01 g, respectively) was added into the medium and cultivated for 10 more days.

Isolation of Metabolites 2 and 3. After the fermentation, culture medium and mycelia were separated by filtration. The medium was acidified to pH 2 with 1 M HCl, saturated with NaCl, and extracted with CH_2Cl_2. The mycelia were also extracted with CH_2Cl_2. Both CH_2Cl_2 extracts were mixed, the solvent was evaporated, and crude extract (1.98 g) was obtained. The extract was suspended in CH_2Cl_2 (1 L) and partitioned between CH_2Cl_2 and 5% aqueous $NaHCO_3$ (200 mL). The solvent of the CH_2Cl_2 layer was evaporated to give the neutral part (1.68 g). The water layer was acidified to pH 2 with 1 M HCl, saturated with NaCl, and extracted with CH_2Cl_2. The solvent (CH_2Cl_2) of the 5% aqueous $NaHCO_3$ soluble part was evaporated to give the acidic part (0.14 g). The neutral part was chromatographed on Si-60 columns with a hexane−EtOAc gradient (19:1 to 0:20). Substrate **1** (285 mg) (R_f = 0.70, S1) and metabolites **2** (580 mg) (R_f = 0.58, S2), and **3** (106 mg) (R_f = 0.38, S2) were isolated.

Compound **2**: oil; HRFAB-MS (pos), *m/z* 241.2141 $[MH]^+$, calcd for $C_{15}H_{29}O_2$, 241.2168; IR ν_{max} 3402, 2971, 2939, 1461, 1377, 1199, 1152, 996, 919 cm^{-1}; ^1H NMR see Table 2; ^{13}C NMR see Table 3.

Compound **3**: oil; FAB-MS (neg), *m/z* 255 $[M - H]^-$; IR ν_{max} 3420, 2972, 2933, 1451, 1383, 1159, 1078, 996, 923 cm^{-1}; ^1H NMR see Table 2; ^{13}C NMR see Table 3.

Biotransformation of Nerylacetone (4) for 7 Days. Precultured *G. cingulata* (10 mL) was transferred into a 3 L stirred fermentor containing 1.2 L of medium. Cultivation was carried out at 27 °C with stirring (ca. 100 rpm) for 3 days under aeration (200 mL min^{-1}). After the growth of *G. cingulata*, **4** (2.56 g) was added into the medium and cultivated for 7 more days.

Isolation of Metabolites 5−9. After the fermentation, culture medium and mycelia were separated by filtration. The medium was acidified to pH 2 with 1 M HCl, saturated with NaCl, and extracted with CH_2Cl_2. The mycelia were also extracted with CH_2Cl_2. Both CH_2Cl_2 extracts were mixed, the solvent was evaporated, and crude extract (2.47 g) was obtained. The extract was separated to the neutral part (2.20 g) and the acidic part (0.15 g) in the same manner as described above. The neutral part was chromatographed on Si-60 columns with a hexane−EtOAc gradient (19:1 to 0:20), and substrate **4** (190 mg) (R_f = 0.72, S1) and metabolites **5** (285 mg) (R_f = 0.36, S2), **6** (40 mg) (R_f = 0.60, S1), **7** (290 mg) (R_f = 0.58, S2), **8** (105 mg) (R_f = 0.26, S2), and **9** (204 mg) (R_f = 0.45, S2) were isolated.

Compound **5**: oil; FAB-MS (pos), *m/z* 229 $[MH^+]$; IR ν_{max} 3424, 2972, 2939, 1709, 1363, 1163, 1079 cm^{-1}; ^1H NMR see Table 4; ^{13}C NMR see Table 5.

Compound **6**: oil; EI-MS, *m/z* 196 $[M]^+$; IR ν_{max} 3354, 2967, 2925, 2857, 1451, 1376, 1128 cm^{-1}; ^1H NMR see Table 4; ^{13}C NMR see Table 5.

Compound **7**: oil; HRFAB-MS (pos), *m/z* 213.1867 $[MH^+]$, calcd for $C_{13}H_{25}O_2$, 213.1855; IR ν_{max} 3425, 2970, 2939, 1713, 1363, 1158 cm^{-1}; ^1H NMR see Table 4; ^{13}C NMR see Table 5.

Table 1. Relative Abundance of Metabolites Obtained by Oxidation at Remote Double Bond of 1, 4, 10, and 12 with the Fungus *G. cingulata*

remote double bond →	HO (diol)	OH, HO (triol)	residual substrate
trans-nerolidol (**1**)	**70% (2)**	10% (**3**)	15% (**1**)
cis-nerolidol (**10**)	nd[a]	**85% (11)**	10% (**10**)
geranylacetone (**4**)	**53% (7+9)**	29% (**5+8**)	15% (**4**)
nerylacetone (**12**)	12% (**15**)	**60% (13+16)**	15% (**12**)

[a] nd, not detected.

Table 2. ^1H-NMR Spectral Data of Compounds 1−3 (500.00 MHz; Chemical Shifts in ppm; Coupling Constants in Hz)

H	1	2	3
1	5.06 dd (11, 1.5)	5.06 dd (11, 1.5)	5.06 d (11)
	5.21 dd (17.5, 1.5)	5.22 dd (17.5, 1.5)	5.21 d (17.5)
2	5.92 dd (17.5, 11)	5.92 dd (17.5, 11)	5.91 dd (17.5, 11)
4	1.58 m	1.58 m	1.36−1.60 m
5	1.96−2.10 m	1.97 t (7)	2.00−2.26 m
6	5.14 tq (7, 1)	5.15 tq (7, 1)	5.21 m
8	1.96−2.10 m	2.04 m	2.00−2.26 m
9	1.96−2.10 m	1.40−1.48 m	1.36−1.60 m
10	5.08 m	1.40−1.48 m	3.33 d (10.5)
12	1.68 d (1)	1.21 s	1.15 s
13	1.60 s	1.21 s	1.19 s
14	1.60 s	1.60 s	1.61 s
15	1.28 s	1.28 s	1.28 s

Table 3. ^{13}C-NMR Spectral Data of Compounds 1−3 (125.65 MHz; Chemical Shifts in ppm; Multiplicities Determined by the DEPT Pulse Sequence)

C	1	2	3
1	111.60 (CH_2)	111.66 (CH_2)	111.69 (CH_2)
2	145.05 (CH)	145.03 (CH)	144.93 (CH)
3	73.42 (C)	73.45 (C)	73.43 (C)
4	42.05 (CH_2)	42.08 (CH_2)	41.90 (CH_2)
5	22.68 (CH_2)	22.67 (CH_2)	22.68 (CH_2)
6	124.23 (CH)	124.39 (CH)	124.93/124.9 (CH)
7	135.47 (C)	135.43 (C)	135.18 (C)
8	39.65 (CH_2)	39.96 (CH_2)	36.71/36.68 (CH_2)
9	26.61 (CH_2)	22.58 (CH_2)	29.60 (CH_2)
10	124.20 (CH)	43.44 (CH_2)	78.08/78.05 (CH)
11	131.33 (C)	70.96 (C)	73.00 (C)
12	25.62 (Me)	29.20 (Me)	26.32 (Me)
13	17.62 (Me)	29.20 (Me)	23.16 (Me)
14	15.96 (Me)	15.87 (Me)	15.87 (Me)
15	27.80 (Me)	27.81 (Me)	27.80/27.75 (Me)

Table 4. ^{1}H-NMR Spectral Data of Compounds 4−9 (4, 5, 7: 500.00 MHz; 6, 8: 270.05 MHz; Chemical Shifts in ppm; Coupling Constants in Hz)

H	4	5	6	7	8	9
1	2.13 s	2.14 s	1.19 d (6)	2.14 s	1.18 d (7)	1.18 d (6)
2			3.81 sextet (6)		3.80 m	3.80 sextet (6)
3	2.46 t (7.5)	2.47 t (7.5)	1.47−1.52 m	2.47 t (7.5)	1.36−1.62 m	1.38−1.54 m
4	2.26 m	2.27 m	1.97−2.12 m	2.27 m	2.05−2.28 m	1.94−2.12 m
5	5.08 m	5.15 m	5.14 m	5.08 m	5.22 t (7)	5.15 m
7	1.92−2.08 m	1.22−1.61 m	1.97−2.12 m	1.97 t (6.5)	2.05−2.28 m	1.94−2.12 m
8	1.92−2.08 m	1.22−1.61 m	1.97−2.12 m	1.40−1.52 m	1.36−1.62 m	1.38−1.54 m
9	5.08 m	3.33 dd (10.5, 2)	5.08 m	1.40−1.52 m	3.33 d (10.5)	1.38−1.54 m
11	1.68 d (1)	1.20 s	1.68 d (1)	1.21 s	1.15 s	1.20 s
12	1.61 s	1.16 s	1.60 s	1.21 s	1.19 s	1.20 s
13	1.59 s	1.63 s	1.62 s	1.61 s	1.63 s	1.61 s

Table 5. ^{13}C-NMR Spectral Data of Compounds 4−9 (4, 5, 7: 125.65 MHz; 6, 8: 67.80 MHz; Chemical Shifts in ppm; Multiplicities Determined by the DEPT Pulse Sequence)

C	4	5	6	7	8	9
1	29.85 (Me)	29.80 (Me)	23.44 (Me)	29.76 (Me)	23.35 (Me)	23.32 (Me)
2	208.75 (C)	209.06 (C)	67.91 (CH)	208.76 (C)	67.81 (CH)	67.68 (CH)
3	43.68 (CH_2)	43.52 (CH_2)	39.17 (CH_2)	43.61 (CH_2)	38.97 (CH_2)	39.91 (CH_2)
4	22.42 (CH_2)	22.30 (CH_2)	24.35 (CH_2)	22.32 (CH_2)	24.35 (CH_2)	22.52 (CH_2)
5	122.48 (CH)	122.96 (CH)	123.94 (CH)	122.56 (CH)	124.68 (CH)	124.08 (CH)
6	136.29 (C)	136.11 (C)	135.65 (C)	136.16 (C)	135.27 (C)	135.40 (C)
7	39.58 (CH_2)	36.57 (CH_2)	39.70 (CH_2)	39.81 (CH_2)	36.68 (CH_2)	39.09 (CH_2)
8	26.55 (CH_2)	29.58 (CH_2)	26.63 (CH_2)	22.40 (CH_2)	29.60 (CH_2)	24.25 (CH_2)
9	124.13 (CH)	72.88 (CH)	124.25 (CH)	43.27 (CH_2)	73.01 (CH)	43.35 (CH_2)
10	131.31 (C)	77.89 (C)	131.38 (C)	70.69 (C)	77.98 (C)	70.85 (C)
11	25.60 (Me)	26.22 (Me)	25.65 (Me)	29.08 (Me)	26.31 (Me)	29.07 (Me)
12	17.59 (Me)	23.12 (Me)	17.65 (Me)	29.08 (Me)	23.15 (Me)	29.10 (Me)
13	15.86 (Me)	15.81 (Me)	15.96 (Me)	15.71 (Me)	15.84 (Me)	15.75 (Me)

Compound **8**: oil; HRFAB-MS (pos), *m/z* 231.1954 [MH^+], calcd for $C_{13}H_{27}O_3$, 231.1961; IR ν_{max} 3354, 2967, 2925, 2857, 1451, 1376, 1128 cm^{-1}; ^{1}H NMR see Table 4; ^{13}C NMR see Table 5.

Compound **9**: oil; HRFAB-MS (pos), *m/z* 215.2021 [MH^+] calcd for $C_{13}H_{27}O_2$, 215.2012; IR ν_{max} 3356, 2969, 2938, 2867, 1460, 1377, 1131 cm^{-1}; ^{1}H NMR see Table 4; ^{13}C NMR see Table 5.

RESULTS AND DISCUSSION

To clarify the time course of the microbial transformation of *trans*-nerolidol (**1**) by *G. cingulata*, a small amount of **1** was incubated with *G. cingulata* for 10 days. Two major metabolites (**2** and **3**) and a small amount of minor metabolites were detected by TLC and GC analysis. The time course of relative abundance of metabolites was qualitatively observed by TLC and quantitatively measured by GC (Figure 2). In this system, the starting substrate **1** was mainly transformed to **2** and **3**, and about 85% of **1** was consumed in 10 days. The major metabolite **2** made up about 70% of the products in 6 days.

To isolate these metabolites, a large-scale incubation of **1** using *G. cingulata* was carried out for 10 days. After the biotransformation, the culture was extracted as described and metabolites **2** and **3** were isolated from the CH_2Cl_2 extract. The structures of these metabolites were determined by spectral data.

Metabolite **2** has a molecular formula of $C_{15}H_{28}O_2$ based on its mass spectrum. Its ^{1}H- and ^{13}C-NMR signals indicated the presence of a trisubstituted double bond [δ_H 5.15, δ_C 124.39 (CH) and 135.43 (C)] bearing a methyl group (δ_H 1.60, δ_C 15.87), a monosubstituted double bond [δ_H 5.06, 5.22, and 5.92, δ_C 111.66 (CH_2) and 145.03 (C)], and two tertiary hydroxyl groups. From the spectral data, metabolite **2** was elucidated to be (*E*)-3,7,11-trimethyl-1,6-dodecadiene-3,11-diol. Metabolite **2** had not been obtained previously by microbial transformation of **1**.

Metabolite **3** has a molecular formula of $C_{15}H_{28}O_3$ based on its mass spectrum. Its ^{1}H- and ^{13}C-NMR signals indicated the presence of a secondary hydroxyl group (δ_H 3.33, δ_C 78.08/78.05), a trisubstituted double bond [δ_H 5.21, δ_C 124.93/124.90 (CH) and 135.18 (C)] bearing a methyl group (δ_H 1.61, δ_C 15.87), a monosubstituted double bond [δ_H 5.06, 5.21, and 5.91, δ_C 111.69 (CH_2) and 144.93 (C)], and two tertiary hydroxy groups. From the spectral data, metabolite **3** was identified to be (*E*)-3,7,11-trimethyl-1,6-dodecadiene-3,10,11-triol (mixture of diastereoisomers). Metabolite **3** had been obtained previously by microbial transformation of **1** (Abraham and Stumpf, 1987; Abraham et al., 1988, 1990; Madyastha and Gurura, 1993). On the biotransformation of **1** by *G. cingulata*, specific hydration at the remote double bond occurred to give **2** as the major metabolite.

To clarify the time course of the microbial transformation of geranylacetone (**4**) by *G. cingulata*, a small amount of **4** was incubated with *G. cingulata*. Five metabolites (**5**−**9**) were detected by TLC and GC analysis. The time course of relative abundance of **4**−**9** was qualitatively observed by TLC and quantitatively measured by GC (Figure 3). In this system, the starting substrate (**4**) was about 85% consumed in 8 days. The major metabolite **5** was about 35% of the products in 6 days.

To isolate these metabolites (**5**−**9**), large-scale incubation of **4** using *G. cingulata* was carried out for 7 days. After the biotransformation, the culture was extracted as described. Metabolites **5**−**9** were isolated from the CH_2Cl_2 extract. The structure of these isolated compounds was determined by spectral data.

Metabolite **5** has a molecular formula of $C_{13}H_{24}O_3$ based on its mass spectrum. Its spectral data indicated the presence of a secondary hydroxyl group (δ_H 3.33, δ_C 72.88), a tertiary hydroxyl group (δ_C 77.89; ν_{max} 3424, 1163 cm^{-1}), a trisubstituted double bond [δ_H 5.15, δ_C 122.96 (CH) and 136.11 (C)] bearing a methyl group (δ_H 1.63; δ_C 15.81), and a carbonyl group (δ_C 209.06; ν_{max} 1709 cm^{-1}). Comparison of spectral data between **5** and metabolites of **4** by other microorganisms (Abraham et

al., 1989; Abraham and Stumpf, 1987; Madyastha and Gurura, 1993; Juttner and Hans, 1986) indicates that **5** is (*E*)-9,10-dihydroxy-6,10-dimethyl-5-undecen-2-one.

Metabolite **6** has a molecular formula of $C_{13}H_{24}O$ based on its mass spectrum. Its spectral data indicated the presence of a secondary hydroxyl group (δ_H 3.81; δ_C 67.91), two trisubstituted double bonds [δ_H 5.14, δ_C 123.94 (CH) and 135.65 (C); and δ_H 5.08; δ_C 124.25 (CH) and 131.38 (C)], and no carbonyl group. From the spectral data, metabolite **6** is elucidated to be (*E*)-6,10-dimethyl-5,9-undecadien-2-ol.

Metabolite **7** has a molecular formula of $C_{13}H_{24}O_2$ based on its mass spectrum. Its spectral data indicated the presence of a tertiary hydroxyl group (δ_C 70.69; ν_{max} 3425, 1158 cm^{-1}), a trisubstituted double bond [δ_H 5.08; δ_C 122.56 (CH) and 136.16 (C)] bearing a methyl group (δ_H 1.61; δ_C 15.71), and a carbonyl group (δ_C 208.76; ν_{max} 1713 cm^{-1}). From the spectral data, metabolite **7** is elucidated to be (*E*)-10-hydroxy-6,10-dimethyl-5-undecen-2-one.

Metabolite **8** has a molecular formula of $C_{13}H_{26}O_3$ based on its mass spectrum. Its spectral data indicated the presence of two secondary hydroxyl groups (δ_H 3.33, δ_C 73.01; and δ_H 3.80, δ_C 67.81), a tertiary hydroxyl group (δ_C 77.98; ν_{max} 3382, 1131 cm^{-1}), a trisubstituted double bond [δ_H 5.22; δ_C 124.68 (CH) and 135.27 (C)] bearing a methyl group (δ_H 1.63; δ_C 15.84), and no carbonyl group. Comparison of spectral data between **8** and **4**−**7** indicates that **8** is elucidated to be (*E*)-6,10-dimethyl-5-undecene-2,9,10-triol.

Metabolite **9** has a molecular formula of $C_{13}H_{26}O_2$ based on its mass spectrum. Its spectral data indicated the presence of a secondary hydroxyl group (δ_H 3.80, δ_C 67.68), a tertiary hydroxyl group (δ_C 70.85; ν_{max} 3356, 1131 cm^{-1}), and no carbonyl group. Comparison of spectral data between **9** and **4**−**8** indicates that **9** is (*E*)-6,10-dimethyl-5-undecene-2,10-diol.

Metabolites **5** and **6** were previously obtained on the biotransformation of **4** by other microorganisms (**5**: Abraham et al., 1989; Abraham and Stumpf, 1987; Madyastha and Gurura, 1993; **6**: Abraham et al., 1989; Juttner and Hans, 1986); however, **7**−**9** had not been obtained on the biotransformation of **4** by other microorganisms. In this system, similarly to **1**, hydration at the remote double bond of **4** was the main metabolic pathway. Hydration at the remote double bond is a characteristic metabolic pathway on the microbial transformation of **1** and **4** by *G. cingulata*.

As shown in Table 1, in the case of **1** and **3** (*trans*-form), hydration of the remote double bond was the main pathway, while in the cases of **10** and **12** (*cis*-form), oxidation of the remote double bond was the main pathway, giving *vic*-diol (Miyazawa et al., 1995). These differences in product formation by *G. cingulata* with the *trans*-form (**1** and **4**) and the *cis*-form (**10** and **12**) may be explained by the influence of the *cis*/*trans* configuration of these substrates. So far, there is no report of a clear distinction between the microbial transformation of the *trans*-form (**1** and **3**) and that of the *cis*-form (**10** and **12**). In other words, *G. cingulata* recognized the *cis*/*trans* configuration on the microbial transformation of **1**, **4**, **10**, and **12**.

LITERATURE CITED

Abraham, W.-R.; Alfmann, H.-A. Addition of water to acyclic terpenoids by *Fusarium solani*. *Appl. Microbiol. Biotechnol.* **1989**, *32*, 295−298.

Abraham, W.-R.; Stumpf, B. Enzymatic acyloin condensation of acyclic aldehydes. *Z. Naturforsch.* **1987**, *42C*, 559−566.

Abraham, W.-R.; Hoffmann, H. M. R.; Kieslich, K.; Reng, G.; Stumpf, B. Microbial transformations of some monoterpenoids and sesquiterpenoids. *Ciba Found. Symp.* **1985**, *111* (*Enzymes Org. Synth.*), 146−150.

Abraham, W.-R.; Stumpf, B.; Alfmann, H.-A. Chiral intermediates by microbial epoxidations. *J. Essent. Oil Res.* **1990**, *2*, 251−257.

Alfmann, H.-A.; Abraham, W.-R.; Kieslich, K. Microbial ω-hydroxylations of *trans*-nerolidol and structurally related sesquiterpenoids. *Biocatalysis* **1988**, *2*, 59−67.

Hashidoko, Y.; Urashima, M.; Yoshida, T.; Mizutani, J. Decarboxylative conversion of hydroxycinnamic acids by *Klebsiella oxytoca* and *Erwinia uredovora*, epiphytic bacteria of *Polymnia sonchifolia* leaf, possibly associated with formation of microflora on the damaged leaves. *Biosci., Biotechnol. Biochem.* **1993**, *57* (2), 215−219.

Holmes, D. S.; Asworth, D. M.; Robinson, J. A. The bioconversion of (3*RS*,*E*)- and (3*RS*, *Z*)-nerolidol into oxygenated products by *Streptomyces cinnamonensis*. Possible implications for the biosynthesis of the polyether antibiotic monensin A? *Helv. Chem. Acta* **1990**, *73*, 260−271.

Juttner, F.; Hans, R. The reducing capacities of cyanobacteria for aldehydes and ketones. *Appl. Microbiol. Biotechnol.* **1986**, *25*, 52−54.

Kieslich, K.; Hoyer, G.-A.; Seeger, A.; Wiechert, R.; Kerb, U. Preparation of several mono- and diketo-structures of D-homo-4-pregnene-3,20-dione and D-homo-1,4-pregnadiene-3,20-dione. *Chem. Ber.* **1980**, *113*, 203−220.

Kuhn, P. J.; Smith, D. A.; Ewing, D. F. 5,7,2′,4′-Tetrahydroxy-8-(3″-hydroxy-3″-methylbutyl)isoflavanone, a metabolite of kievitone produced by *Fusarium solani* f.sp. *phaseoli*. *Phytochemistry* **1977**, *16*, 296−297.

Madyastha, K. M.; Gurura, T. L. Transformations of acyclic isoprenoids by *Aspergillus niger*: selective oxidation of ω-methyl and remote double bonds. *Appl. Microbiol. Biotechnol.* **1993**, *38*, 738−741.

Miyazawa, M.; Nakaoka, H.; Kameoka, H. Biotransformation of 1,8-cineole to (+)-2-*endo*-hydroxy-1,8-cineole by *Glomerella cingulata*. *Chem. Express* **1991**, *6* (9), 667−670.

Miyazawa, M.; Uemura, T.; Kameoka, H. Biotransformation of (−)-globulol and (+)-ledol by *Glomerella cingulata*. *Phytochemistry* **1994**, *37* (4), 1027−1030.

Miyazawa, M.; Nankai, H.; Kameoka, H. Biotransformation of (−)-α-bisabolol by plant pathogenic fungus, *Glomerella cingulata*. *Phytochemistry* **1995a**, *39* (5), 1077−1080.

Miyazawa, M.; Nankai, H.; Kameoka, H. Biotransformation of (+)-cedrol by plant pathogenic fungus, *Glomerella cingulata*. *Phytochemistry* **1995b**, *40* (1), 69−72.

Miyazawa, M.; Nankai, H.; Kameoka, H. Biotransformations of acyclic terpenoids, (±)-*cis*-nerolidol and nerylacetone by plant pathogenic fungus, *Glomerella cingulata*. *Phytochemistry* **1995c**, *40* (4), 1133−1137.

Miyazawa, M.; Suzuki, Y.; Kameoka, H. Biotransformation of (−)-nopol by *Glomerella cingulata*. *Phytochemistry* **1995d**, *39* (2), 337−340.

Miyazawa, M.; Yokote, K.; Kameoka, H. Resolution of racemic linalool oxide-pyranoid by microbial esterification. *Tetrahedron: Asymmetry* **1995e**, *6* (5), 1067−1068.

Tsuda, Y. Microbial reduction of naphthoxypropionic acids. *Chem. Pharm. Bull.* **1987**, *35* (6), 2554−2557.

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