BIOTRANSFORMATION OF ACYCLIC TERPENOID (2E,6E)-FARNESOL BY PLANT PATHOGENIC FUNGUS GLOMERELLA CINGULATA

MITSUO MIYAZAWA,* HIROKAZU NANKAI, HIROMU KAMEOKA

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

Abstract—The microbial transformation of (2E,6E)-farnesol was investigated using the plant pathogenic fungus, *Glomerella cingulata*. At the first step, oxidation proceeded at the remote double bond to give (2E,6E)-3,7,11-trimethyl-2,6-dodecadien-1,11-diol and (2E,6E)-3,7,11-trimethyl-2,6-dodecadien-1,10,11-triol. In the second step, (2E,6E)-3,7,11-trimethyl-2,6-dodecadien-1,11-diol was hydroxylated at the C-5 position and to give (2E,6E)-3,7,11-trimethyl-2,6-dodecadien-1,5,11-triol. In addition, (2E,6E)-3,7,11-trimethyl-2,6-dodecadien-1,5,11-triol was isomerized to (2Z,6E)-3,7,11-trimethyl-2,6-dodecadien-1,5,11-triol. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

We have investigated the microbial transformations of acyclic terpenoids using a plant pathogenic fungus. In our previous papers we reported the microbial transformations of (±)-*cis*-nerolidol (1) [1], nerylacetone (2) [1], (±)-*trans*-nerolidol (3) [2] and geranylacetone (4) [2] by *Glomerella cingulata*. With compounds 1 and 2 (*cis*-form), epoxidation of the remote double bond and subsequent hydrolysis gave (Z)-3,7,11-trimethyl-1,6-dodecadien-3,10,11-triol (from 1) and (Z)-9,10-dihydroxy-6,10-dimethyl-5-undecen-2-one (from 2) as the major metabolites. With compounds 3 and 4 (*trans*-form), hydration of the remote double bond was the main reaction to give (E)-3,7,11-trimethyl-1,6-dodecadien-3,11-diol (from 3) and (E)-10-hydroxy-6,10-dimethyl-5-undecen-2-one (from 4) as major metabolites. This difference in product formation between the *cis*-form (1 and 2) and the *trans*-form (3 and 4) of the substrates by *G. cingulata* can be explained by the influence of the *cis*/*trans* configuration of these substrates.

In order to clarify further the differences in product formation by *C. cingulata* with acyclic terpenoids, the microbial transformation of (2E,6E)-farnesol (5) using *G. cingulata* was investigated. There have been reports about the microbial transformation of 5 [3–5]. Compound 5 was transformed into (2Z,6E)-farnesol by *Helminthosporium sativum* [3], to (2E)-3,7,11-trimethyl-2,10-dodecadien-1,7-diol by *Fusarium oxysporum* [4], to (2E,6E)-3,7,11-trimethyl-2,6,10-dodecatrien-1-carboxylic acid by *Pseudomonas au-*

reofaciens [4] and to (2E,6E)-3,7,11-trimethyl-2,6-dodecadien-1,10,11-triol (7) and (2E,6E)-3,7,11-trimethyl-2,6,10-dodecatrien-1,13-diol by *Aspergillus niger* [5]. So far, (2E,6E)-3,7,11-trimethyl-2,6-dodecadien-1,11-diol (6), (2E,6E)-3,7,11-trimethyl-2,6-dodecadien-1,5,11-triol (8) and (2Z,6E)-3,7,11-trimethyl-2,6-dodecadien-1,5,11-triol (9) have not been observed during the biotransformation of 5. The present paper is the first report of the production of 6, 8 and 9 during the biotransformation of 5.

RESULTS AND DISCUSSION

For time-course experiments on the microbial transformation of 5 by *G. cingulata*, a small amount of 5 was incubated with *G. cingulata* for 12 days. Four major metabolites and minor metabolites were detected by TLC, GC and GC–mass spectral analysis. These metabolites were not detected by TLC or GC analysis of the culture of *G. cingulata* to which no substrate (5) was fed, nor were they produced in a mixture of 5 and the medium which was stirred for 12 days. It was demonstrated, therefore, that *G. cingulata* transformed 5 into various metabolites. The time-course of relative concentration changes of substrate (5) and metabolites was monitored by TLC and quantitatively measured by a GC method (Fig. 1). The starting substrate 5 was completely consumed after two days. Metabolite 6 decreased after one day, and 6 completely disappeared by four days. Metabolite 7 decreased after two days, but metabolites 8 and 9 increased gradually. In addition, many minor metabolites appeared after eight days and these products may be highly degraded metabolites.

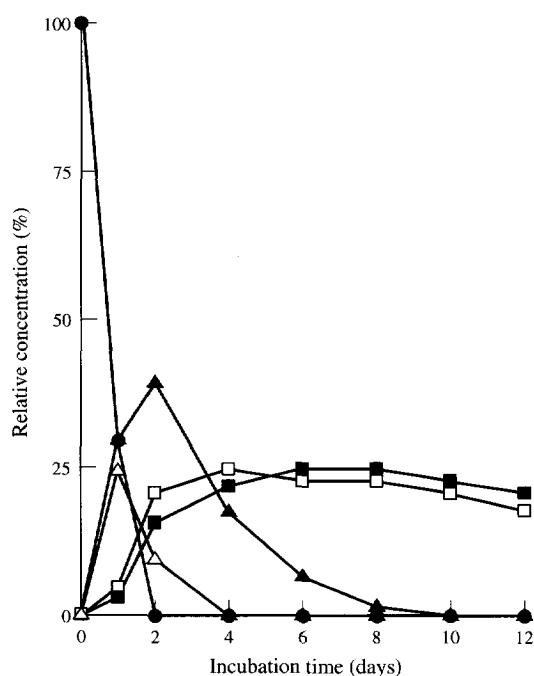*Author to whom correspondence should be addressed.

Fig. 1. Time course of the transformation of compound 5 by G. cingulata: ● = (2E,6E)-farnesol (5); △ = (2E,6E)-3,7,11-trimethyl-2,6-dodecadren-1,11-diol (6); ▲ = (2E,6E)-3,7,11-trimethyl-2,6-decadien-1,10,11-triol (7); □ = (2E,6E)-3,7,11-trimethyl-2,6-dodecadien-1,5,11-triol (8); ■ = (2Z,6E)-3,7,11-trimethyl-2,6-dodecadien-1,5,11-triol (9).

In order to isolate metabolites 6 and 7 a large scale incubation of 5 using G. cingulata was carried out for one day. After the biotransformation the culture was extracted and metabolites 6 and 7 were isolated from the crude extract. The structures of 6 and 7 were then determined by spectral analysis.

The metabolite 6 had a molecular formula $C_{15}H_{28}O_2$ based on its EI-mass spectrum. Other spectral data indicated the presence of a primary hydroxyl group (δ_H 4.15; δ_C 59.3; ν_{max} 3333 and 1009 cm^{-1}), a tertiary hydroxyl group (δ_C 71.1; ν_{max} 3333, 1150 cm^{-1}) and two trisubstituted double bonds [(δ_H 5.40; δ_C 124.1 (CH) and 139.0 (C)) and (δ_H 5.10; δ_C 123.8 (CH) and 135.0 (C))] each bearing one methyl group [(δ_H 1.59; δ_C 16.1) and (δ_H 1.67; δ_C 15.8)]. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) were assigned by comparison with the spectral data for 5 and metabolites of 1–4 produced by G. cingulata [1, 2]. Based on the spectral data, the metabolite 6 was elucidated to be (2E,6E)-3,7,11-trimethyl-2,6-dodecadien-1,11-diol.

The metabolite 7 had a molecular formula $C_{15}H_{28}O_3$ based on its FAB-mass spectrum. Other spectral data indicated the presence of a primary hydroxyl group (δ_H 4.14; δ_C 59.0; ν_{max} 3370, 998 cm^{-1}), a secondary hydroxyl group (δ_H 3.36; δ_C 77.4; ν_{max} 3370 and 1076 cm^{-1}), a tertiary hydroxyl group (δ_C 73.1; ν_{max} 3370 and 1158 cm^{-1}) and two trisubstituted double bonds [(δ_H 5.39; δ_C 124.1 (CH) and 138.4 (C)) and (δ_H 5.17; δ_C 124.9 (CH) and 134.8 (C))] each bearing

one methyl group [(δ_H 1.62; δ_C 15.8) and (δ_H 1.65; δ_C 15.7)]. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) were assigned by comparison with the spectral data for 5, 6 and metabolites of 1–4 produced by G. cingulata [1, 2]. Based on the spectral data, the metabolite 7 was identified as (2E,6E)-3,7,11-trimethyl-2,6-dodecadien-1,10,11-triol.

In order to confirm the structures of 8 and 9 a large scale incubation of 5 using G. cingulata was conducted for seven days. After the biotransformation the culture was extracted and metabolites 8 and 9 were isolated from the crude extract. The structures of 8 and 9 were determined from their spectral data.

The metabolite 8 had a molecular formula $C_{15}H_{28}O_3$ based on its FAB-mass spectrum. Other spectral data indicated the presence of a primary hydroxyl group (δ_H 4.15; δ_C 59.0; ν_{max} 3361, 1006 cm^{-1}), a secondary hydroxyl group (δ_H 4.52; δ_C 66.2; ν_{max} 3361 and 1035 cm^{-1}), a tertiary hydroxyl group (δ_C 70.9; ν_{max} 3361 and 1151 cm^{-1}) and two trisubstituted double bonds [(δ_H 5.48; δ_C 127.0 (CH) and 135.4 (C)) and (δ_H 5.18; δ_C 127.6 (CH) and 138.1 (C))] each bearing one methyl group [(δ_H 1.68; δ_C 16.5) and (δ_H 1.72; δ_C 16.4)]. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) were assigned by comparison with the spectral data for compounds 5–7 and metabolite 8 was shown to be (2E,6E)-3,7,11-trimethyl-2,6-dodecadien-1,5,11-triol. The chemical shift values observed in the ^{1}H NMR spectrum of 8a (acetylated compound derived from 8) further confirmed the structure of 8. The ^{1}H NMR spectrum of 8a displayed three signals at δ 1.97, 2.01 and 2.05 due to the triacetate; other signals are summarized in Table 1.

The metabolite 9 had a molecular formula $C_{15}H_{28}O_3$ based on its FAB-mass spectrum. Other spectral data indicated the presence of a primary hydroxyl group (δ_H 3.95 and 4.16; δ_C 57.8; ν_{max} 3333 and 1003 cm^{-1}), a secondary hydroxyl group (δ_H 4.49; δ_C 65.7; ν_{max} 3333 and 1045 cm^{-1}), a tertiary hydroxyl group (δ_C 70.9; ν_{max} 3333 and 1153 cm^{-1}) and two trisubstituted double bonds [(δ_H 5.72; δ_C 127.7 (CH) and 138.1 (C)) and (δ_H 5.26; δ_C 16.9 (CH) and 137.4 (C))] each bearing one methyl group [(δ_H 1.69; δ_C 16.4) and (δ_H 1.82; δ_C 23.9). The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) were assigned by comparison with the spectral data for compounds 5–8 and metabolite 9 was thus elucidated to be (2Z,6E)-3,7,11-trimethyl-2,6-dodecadien-1,5,11-triol(geometrical isomer of 8). The chemical shift values observed in the ^{1}H NMR spectrum of 9a (acetylated compound derived from 9) further confirmed the structure of 9. The ^{1}H NMR spectral data of 9a displayed three signals at δ 1.97, 2.01 and 2.05 due to the triacetate, and other signals are summarized in Table 1.

The metabolic pathways for the biotransformation of 5 by G. cingulata (Scheme 1) were derived from the time-course experiment (Fig. 1) and the structures of the metabolites. The reactions were hydration (5→6) or epoxidation and subsequently hydrolysis (5→7). Metabolite 8 was then formed, not by hydration of

Table 1. ¹H NMR spectral data for compounds **5**–**9** (270.05 Mhz, CDCl₃, TMS as int. standard)

| H	5	6	7	8	8a	9	9a
1	}4.15 d (7)	}4.15 d (7)	}4.14 d (7)	}4.15 d (7)	}4.55 d (7)	3.95 dd (12, 7)	4.55 dd (13, 7)
1'						4.16	4.63 dd (13, 7)
2	5.42 t (7)	5.40 t (7)	5.39 t (7)	5.48 t (7)	5.37 t (7)	5.72 t (7)	5.44 t (7)
4	}1.95–2.15 m	}1.95–2.15 m	}2.07–2.20 m	2.16 dd (13.5, 5.5)	2.19 dd (13.5, 6)	2.02 dd (13, 4)	2.20 dd (14, 6)
4'				2.26 dd (13.5, 8)	2.39 dd (13.5, 8)	2.56 dd (13, 9)	2.56 dd (14, 8)
5	}1.95–2.15 m	}1.95–2.15 m	}2.07–2.20 m	4.52 ddd (8, 8, 5.5)	5.66 ddd (9, 8, 6)	4.49 ddd (9, 9, 4)	5.64 ddd (9, 8, 6)
5'				—	—		—
6	5.15 m	5.10 t (7)	5.17 t (7)	5.18 d (8)	5.11 d (9)	5.26 d (9)	5.12 d (9)
8	}1.95–2.15 m	}1.95–2.15 m	}2.07–2.20 m	}1.98–2.05 m	}1.94–2.02 m	}1.98–2.05 m	}1.95–2.02 m
8'							
9	}1.95–2.15 m	}1.41–1.45 m	1.41 m	}1.40–1.50 m	}1.62–1.67 m	}1.40–1.52 m	}1.62–1.70 m
9'							
10	5.09 m	1.41–1.45 m	3.36 dd (11, 2)	1.40–1.50 m	1.62–1.67 m	1.40–1.52 m	1.62–1.70 m
10'	—		—				
12	1.68 s	1.21 s	1.14 s	1.20 s	1.42 s	1.22 s	1.42 s
13	1.60 s	1.21 s	1.18 s	1.21 s	1.42 s	1.22 s	1.42 s
14	1.60 s	1.59 s	1.62 s	1.69 d (1)	1.70 (d) (1)	1.69 d (1)	1.71 d (1)
15	1.68 s	1.67 s	1.65 s	1.72 s	1.74 s	1.82 s	1.80 d (1)
OAc					1.97 s		1.97 s
OAc					2.01 s		2.01 s
OAc					2.05 s		2.05 s

Chemical shifts in ppm; coupling constants in Hz.

Table 2. ^{13}C NMR spectral data for compounds 5–9 (67.80 MHz, CDCl$_3$, residual CHCl$_3$ used as int. ref., $\delta = 77.00$)

C	5	6	7	8	9
1	59.2 (CH$_2$)	59.3 (CH$_2$)	59.0 (CH$_2$)	59.0 (CH$_2$)	57.8 (CH$_2$)
2	123.4 (CH)	124.1 (CH)	124.1* (CH)	127.0* (CH)	127.7* (CH)
3	139.5 (C)	139.0 (C)	138.4 (C)	135.4† (C)	138.1† (C)
4	39.6 (CH$_2$)	39.8 (CH$_2$)	39.2 (CH$_2$)	47.8 (CH$_2$)	40.0‡ (CH$_2$)
5	26.3 (CH$_2$)	25.9 (CH$_2$)	25.5 (CH$_2$)	66.2 (CH)	65.7 (CH)
6	123.8 (CH)	123.8 (CH)	124.9* (CH)	127.6* (CH)	126.9* (CH)
7	135.3 (C)	135.0 (C)	134.8 (C)	138.1† (C)	137.4† (C)
8	39.5 (CH$_2$)	39.4 (CH$_2$)	36.4 (CH$_2$)	39.7 (CH$_2$)	39.7‡ (CH$_2$)
9	26.7 (CH$_2$)	22.5 (CH$_2$)	29.0 (CH$_2$)	22.2 (CH$_2$)	22.2 (CH$_2$)
10	124.3 (CH)	43.2 (CH)	77.4 (CH)	43.1 (CH)	43.2 (CH)
11	131.2 (C)	71.1 (C)	73.1 (C)	70.9 (C)	70.9 (C)
12	25.6 (Me)	29.1 (Me)	26.2† (Me)	29.0‡ (Me)	29.2§ (Me)
13	17.6 (Me)	29.1 (Me)	23.0† (Me)	29.2‡ (Me)	29.2§ (Me)
14	16.2 (Me)	16.1 (Me)	15.8‡ (Me)	16.5§ (Me)	16.4 (Me)
15	15.9 (Me)	15.8 (Me)	15.7‡ (Me)	16.4§ (Me)	23.9 (Me)

Chemical shifts in ppm; multiplicities were determined by DEPT pulse sequence.
*,†,‡,§Values are interchangeable within each column.
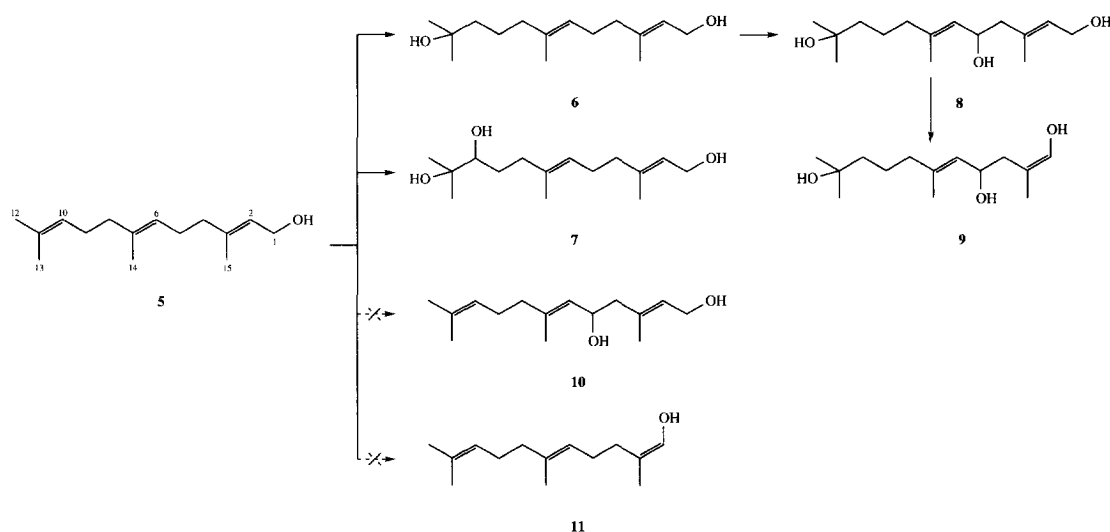
Scheme 1. Metabolic pathways of compound 5 by *G. cingulata*.

(2*E*,6*E*)-5-hydroxyfarnesol (10), but via hydroxylation at the C-5 position of 6, because 10 was not detected in this experiment. In addition, metabolite 9 was formed not via (2*Z*,6*E*)-farnesol (11), but via 8, because of 11 was not detected. Similar to the biotransformation of other acyclic terpenoids (1–4) by *G. cingulata*, compound 5 was oxidized first at the remote double bond to give 6 and 7. In addition, hydroxylation at the C-5 position of 6 and isomerization at the C-2 position of 8 were observed. Metabolites 6, 8 and 9 were not obtained by other biotransformations of 5. This is the first report of the hydration at the remote double bond of 5 and subsequently hydroxylation at the C-5 position of 6. As regards isomerization of the 2,3-double bond of 5 and its derivatives, there have been reports that 5 and (2*E*,6*E*)-10,11-epoxyfarnesol are isomerized to the (2*Z*,6*E*)-form by *Helminthosporium sativum* [3]. However, in the microbial transformation of 5 by *G.*

cingulata, isomerization of 5 to (2*Z*,6*E*)-farnesol was not seen. After hydroxylation at the C-5 position of 6, isomerization of the 2,3-double bond of 8 to the (2*Z*,6*E*)-form proceeded in order to give 9. This isomerization is also noted for the first time during the microbial transformation of 5.

EXPERIMENTAL

Preculture of Glomerella cingulata. *G. cingulata* were precultured by the same procedure described in a previous paper [1].

Time-course experiment. Precultured *G. cingulata* was transferred into a 100 ml Erlenmeyer flask containing 50 ml of medium, and stirred for 3 days. After the growth of *G. cingulata*, compound 5 (50 mg) was added to the medium and the organism cultivated 12 more days. Every day, 5 ml of the culture medium was

removed, satd with NaCl, extracted with EtOAc and the solvent then evapd. The crude extract was analysed by TLC, GC and GC-MS. The relative concns of substrate (**5**) and metabolites were determined on the basis of GC peak area (Fig. 1).

Large-scale biotransformation of **5** *for 1 day.* Precultured *G. cingulata* was transferred into a 1 l stirred fermentor containing 500 ml of medium. Cultivation was carried out at 27° with stirring for 3 days and under aeration. After the growth of *G. cingulata*, compound **5** (500 mg) was added to the medium and then cultivation continued for 1 more day.

Isolation of metabolites **6** *and* **7**. After fermentation the culture medium and mycelium were sepd by filtration. The medium was satd with NaCl and extracted with CH_2Cl_2. The mycelium was also extracted with CH_2Cl_2. The CH_2Cl_2 extracts were combined and the solvent was evapd to yield a crude extract (480 mg). The extract was sepd into neutral (425 mg) and the acidic parts (15 mg) in the usual manner. The neutral part was subjected to CC Si-60 with hexane–EtOAc stepwise from 19:1 to 1:9. Substrate **5** (140 mg), metabolites **6** (70 mg) and metabolite **7** (80 mg) were isolated.

Compound **6**. Oil; EIMS *m/z* 240 [M]$^+$; IR ν_{max} cm^{-1}: 3333, 2938, 1666, 1440, 1381, 1203, 1150, 1009; ^{1}H NMR: see Table 1; ^{13}C NMR; see Table 2.

Compound **7**. Oil; FABMS (positive ion) *m/z* 257 [MH]$^+$; IR ν_{max} cm^{-1}: 3370, 2979, 1669, 1456, 1385, 1158, 1076, 998; ^{1}H NMR; see Table 1; ^{13}C NMR: see Table 2.

Biotransformation of **5** *for 7 days.* Precultured *G. cingulata* was transferred into a 1 l stirred fermentor containing 500 ml of medium. Cultivation was carried out at 27° with stirring for 3 days under aeration. After

the growth of *G. cingulata*, compound **5** (500 mg) was added to the medium and the organism cultivated for 7 more days.

Isolation of metabolites **8** *and* **9**. After fermentation the culture medium and mycelium were sepd by filtration. The medium was satd with NaCl and extracted with CH_2Cl_2. The mycelium was also extracted with CH_2Cl_2. The CH_2Cl_2 extracts were combined and the solvent was evapd to leave a crude extract (465 mg). The extract was sepd into neutral (410 mg) and the acidic parts (25 mg) in the usual manner. The neutral part was subjected to CC Si-60 with hexane–EtOAc stepwise from 19:1 to 1:9. Metabolites **8** (40 mg) and **9** (45 mg) were isolated.

Compound **8**. Oil; FABMS (positive ion) *m/z* 257 [MH]$^+$; IR ν_{max} cm^{-1}: 3361, 2972, 1666, 1443, 1381, 1204, 1151, 1006; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2.

Compound **9**. Oil; FABMS (positive ion) *m/z* 257 [MH]$^+$; IR ν_{max} cm^{-1}: 3333, 2970, 1668, 1445, 1380, 1206, 1153, 1045, 1003; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2.

REFERENCES

1. Miyazawa, M., Nankai, H. and Kameoka, H. (1995) *Phytochemistry* **40**, 1133.
2. Miyazawa, M., Nankai, H. and Kameoka, H. (1996) *J. Agric. Food Chem.* (in press).
3. Suzuki, Y. and Marumo, Y. (1972) *Tetrahedron Letters* 5101.
4. Nakashima, R., Saitou, M., Mizutani, T. and Yamamoto, J. (1992) *Chem. Express* **7**, 489.
5. Madyastha, K. M. and Gurura, T. L. (1993) *Appl. Microbiol. Biotechnol.* **38**, 738.