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BIOTRANSFORMATION OF (2*Z*,6*Z*)-FARNESOL BY THE PLANT PATHOGENIC FUNGUS *GLOMERELLA CINGULATA*

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Key Word Index—*Glomerella cingulata*; microbial transformation; biotransformation: (2*Z*,6*Z*)-farnesol; (2*Z*,6*Z*)-3,7,11-trimethyl-2,6,-dodecadiene-1.10,11-triol; (2*E*,6*Z*)-3,7,11-trimethyl-2,6-dodecadiene-1,10,11-triol; (*Z*)-9,10-dihydroxy-6,10-dimethyl-5-undecen-2-one.

Abstract—The microbial transformation of (2*Z*,6*Z*)-farnesol was investigated using the plant pathogenic fungus, *Glomerella cingulata* as a biocatalyst. Oxidation of the remote double bond and isomerization of the 2,3-double bond gave (2*Z*,6*Z*)-3,7,11-trimethyl-2,6-dodecadiene-1,10,11-triol and (2*E*,6*Z*)-3,7,11-trimethyl-2,6-dodecadiene-1,10,11-triol as major metabolites. One of the further degraded compounds, (*Z*)-9,10-dihydroxy-6,10-dimethyl-5-undecen-2-one, was also obtained.

INTRODUCTION

We previously reported on the microbial transformations of acyclic terpene alcohols and related compounds i.e. (±)-*cis*-nerolidol (**5**) [1], nerylacetone (**6**) [1], (±)-*trans*-nerolidol (**7**) [2], geranylacetone (**8**) [2], (2*E*,5*E*)-farnesol (**9**)[3], citronellol (**10**) [4], citronellal (**11**) [5] and (±)-lavandulol (**12**) [6] by *Glomerella cingulata*. These compounds were mainly oxidized at the remote double bond regioselectively. However, the major metabolic pathways of the *cis*-isomers (**5** and **6**) were different from those of the *trans*-isomers (**7** and **8**). Thus on biotransformation of **5** and **6** by *G. cingulata*, epoxidation of the remote double bond and subsequent hydrolysis to the *vic*-diol was observed as the major metabolic pathway. While on the cases of **7** and **8**, hydration of the remote double bond was the main route. This difference may be explained by the influence of the *E*/*Z* configuration of these substrates. In the case of **9**, the hydrated compounds were also obtained in greater yield than the *vic*-diol. The double bonds of **10** and **11** were oxidized to *vic*-diols. Compound **12** was transformed to (−)-(2*S*,4*S*)-1,5-epoxy-5-methyl-2-(1-methylethenyl)-4-hexanol. To clarify further the influence of the *E*/*Z* configuration on the microbial transformation of acyclic terpenoids, the microbial transformation of (2*Z*,6*Z*)-farnesol (**1**) using *G. cingulata* was investigated.

There had been no reports about the biotransformation of **1**. With regard to the microbial transformation of **9** or mixtures of isomers of farnesols, some investigations were reported (**9**: [3, 7–9], mixture of (2*E*,6*E*)- and (2*Z*,6*E*)-form: [10], *cis* and *trans* mixture: [11], mixture of 4 isomers: [12]). Farnesol (*cis* and *trans* mixture) was transformed to farnesal by *Arthrobacter* sp. [11]. A mixture of four isomers (including **1**) was transformed to 12-hydroxyfarnesol by *Aspergillus niger* [12], but the geometrical configurations were ambiguous. 11-Hydroxygeranylacetone and nerylacetone were obtained on the microbial transformation of farnesol (mixture of 4 isomers including **1**) by *Rhodococcus rubropertinctus* [12]. The present paper is the first report about the microbial transformation of **1**.

RESULTS AND DISCUSSION

To investigate the capacity of *G. cingulata* to metabolize **1**, a time-course experiment was carried out as described in Experimental. Three major metabolites and many minor metabolites were detected by TLC, GC and GC-MS analysis. These metabolites were not detected by TLC or GC analysis of the culture of *G. cingulata* to which no substrate (**1**) was added, nor were they produced in a mixture of **1** and the medium that had been stirred for 10 days. It was demonstrated, therefore, that *G. cingulata* transformed **1** into various metabolites. The time-course of the relative concentration changes of **1** and its metabolites was monitored by TLC and quantitatively measured by GC

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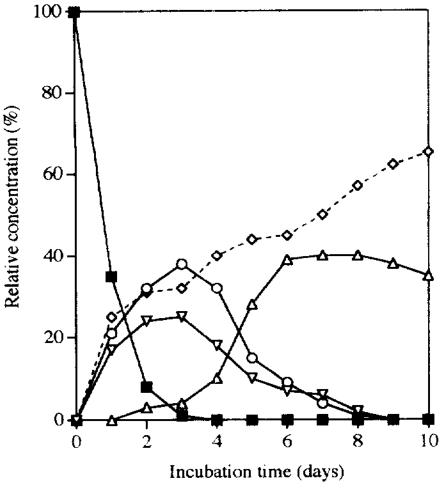


Fig. 1. Time course of the microbial transformation of compound **1** by *G. cingulata*: ■ = (2*Z*,6*Z*)-farnesol (**1**); ○ = (2*Z*,6*Z*)-3,7,11-trimethyl-2,6-dodecadiene-1,10,11, triol (**2**); ▽ = (2*E*,6*Z*)-3,7,11-trimethyl-2,6-dodecadiene-1,10,11-triol (**3**); △ = 9,10-dihydroxynerylacetone (**4**); ◇ = total amounts of unknown minor products.

analysis (Fig. 1). In this system, the starting substrate **1** was completely consumed after four days. Metabolites **2** and **3** decreased after three days, and were completely consumed after nine days. While metabolite **4** increased after two days. In addition, many minor metabolites were detected. These minor products may be highly degraded metabolites.

To isolate metabolite **2**–**4**, incubation of **1** (500 mg) with *G. cingulata* was carried out for four days. After the biotransformation, the culture was extracted as described in the Experimental and metabolites **2**–**4** were isolated from the CH_2Cl_2 extract using chromatography. The structures of **2**–**4** were determined by spectroscopic methods. Metabolite **2** was assigned the molecular formula $C_{15}H_{28}O_3$ based on its mass spectrum. Other spectral data indicated the presence of a primary hydroxyl group (δ_H 4.09; δ_C 58.66; ν_{max} 3374, 997 cm^{-1}), a secondary hydroxyl group (δ_H 3.31; δ_C 78.13; ν_{max} 3374, 1079 cm^{-1}), a tertiary hydroxyl group (δ_C 73.02; ν_{max} 3374, 1164 cm^{-1}) and two trisubstituted double bonds [(δ_H 5.44; δ_C 124.01(CH) and 140.70(C)) and (δ_H 5.19; δ_C 125.37(CH) and 135.63(C))] bearing one methyl group respectively (δ_H 1.70 and 1.75). The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) were assigned by comparison with the data for the metabolites of **9** produced by *G. cingulata* [3]. Based on the spectral data, metabolite **2** was elucidated to be a novel compound, (2*Z*,6*Z*)-3,7,11-trimethyl-2,6-dodecadiene-1,10,11-triol. Compound **2** showed a specific rotation of +3.3°. While (2*E*,6*E*,10*S*)-3,7,11-trimethyl-2,6-dodecadiene-1,10,11-triol, the geometrical isomer of **2**, showed a specific rotation of −10.2° [13]. From the specific rotation values, **2** may be racemate.

Metabolite **3** ($[\alpha]^D_{20}$ +0.7°) was assigned the molecular formula $C_{15}H_{28}O_3$ based on its mass spectrum. Other spectral data indicated the presence of a primary hydroxyl group (δ_H 4.15; δ_C 59.32; ν_{max} 3381, 999 cm^{-1}), a secondary hydroxyl group (δ_H 3.33; δ_C 78.33; ν_{max} 3381, 1079 cm^{-1}), a tertiary hydroxyl group (δ_C 73.04; ν_{max} 3381, 1163 cm^{-1}) and two trisubstituted double bonds [(δ_H 5.44; δ_C 123.54(CH) and 139.52(C)) and (δ_H 5.16; δ_C 125.41(CH) and 135.13(C))] each bearing one methyl group (δ_H 1.66 and 1.70). The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) were assigned by comparison with the spectral data for **2** and metabolite **3** was shown to be a novel compound, (2*E*,6*Z*)-3,7,11-trimethyl-2,6-dodecadiene-1,10,11-triol. The specific rotation indicated compound **3** may be racemate.

The metabolite **4** was identified as (*Z*)-9,10-dihydroxy-6,10-dimethyl-5-undecen-2-one, which is one of the metabolites formed by microbial transformation of **6** by *G. cingulata* [1].

In agreement with other biotransformations by *G. cingulata* [1–6], regioselective oxidation of the remote double bond was observed (Scheme 1). The epoxidation of the remote double bond and subsequent hydrolysis gave compound **2** although the intermediate epoxide could not be detected. In addition, isomerization of the 2,3-double bond led to compound **3**. However (2*E*,6*Z*)-farnesol was not detected during this biotransformation. Therefore the microbial transformation of **2** and **3** by *G. cingulata* was investigated. After the microbial transformation of **2** for 36 hr, residual substrate **2** (55%), and metabolites **3** (33%) and **4** (2%) were detected by GC and GC-MS. While in the case of **3**, the residual substrate **3** (59%), and metabolites **2** (28%) and **4** (2%) were identified. From this result, the main pathway of isomerization of the 2*Z*-form to the 2*E*-form is not **1** → (2*E*,6*Z*)-farnesol but **2** → **3**. Compound **2** came to equilibrium with compound **3** during this biotransformation, then both **2** and **3** were further transformed and degraded by prolonged incubation. One of the degraded compounds was identified to be compound **4**.

In the other paper on the microbial transformation of farnesol (mixture of 4 isomers), nerylacetone was obtained as a major metabolite [12]. However, compound **4** had not been seen on the biotransformations of farnesols. The isomerizations (**2** → **3** and **3** → **2**) is also noted for the first time.

Unlike the biotransformation of **9** by *G. cingulata* [3], in this study, a hydrated compound was not obtained. Such a difference was also reported in our previous study [1, 2], and this difference may be explained by the influence of the *E*/*Z* configuration of these substrates.

EXPERIMENTAL

Preparation of (2Z,6Z)*-farnesol* 1

(2*Z*,6*Z*)-Farnesol (**1**) was isolated from mixture of four isomers by chromatography on silver nitrate (5%) impregnated silica gel [14].

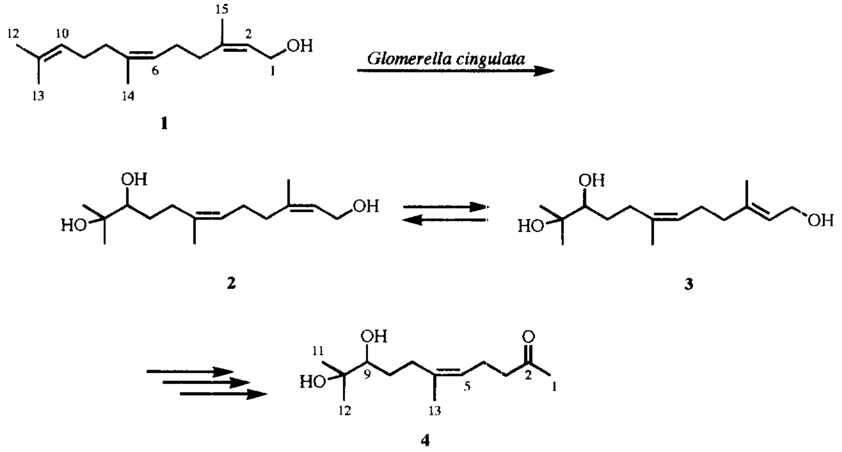
Table 1. ^{1}H NMR spectral data for compounds **1**–**4** (**1**–**3**; 500.00 MHz, **4**; 270.05 MHz, $CDCl_3$, TMS as int. standard).

H	**1**	**2**	**3**	**4**
1	4.09 *d* (7)	4.09 *br d* (7)	4.15 *br d* (7)	2.14 *s*
2	5.45 *dt* (1.5, 7)	5.44 *tq* (7,1)	5.43 *tq* (7,1)	
3	—		—	2.50 *t* (7)
4	2.00–2.12 *m*	2.05–2.30 *m*	2.02–2.28 *m*	2.19–2.44 *m*
5	2.00–2.12 *m*	2.05–2.30 *m*	2.02–2.28 *m*	5.08 *t* (7)
6	5.11 *m*	5.19 *t* (7)	5.16 *t* (7)	
7	—	—	—	2.19–2.44 *m*
8	2.00–2.12 *m*	2.05–2.30 *m*	2.02–2.28 *m*	1.39 *m*
8′	2.00–2.12 *m*	2.05–2.30 *m*	2.02–2.28 *m*	1.60 *m*
9	2.00–2.12 *m*	1.34 *dddd* (14,10.5,9,5)	1.39 *dddd* (14,10.5,8.5,5.5)	3.31 *dd* (2.11)
9′	2.00-2.12 *m*	1.59 *dddd* (14,9.5,7,2)	1.56 *dddd* (14,9,7,2)	—
10	5.11 *m*	3.31 *br d* (10.5)	3.33 *dd* (2,10.5)	
11	—	—	—	1.16 *s*
12	1.69 *s*	1.15 *s*	1.16 *s*	1.21 *s*
13	1.61 *s*	1.19 *s*	1.20 *s*	1.68 *d* (1)
14	1.69 *s*	1.70 *d* (1)	1.66 *s*	
15	1.75 *d* (1.5)	1.75 *d* (1)	1.70 *d* (1)	—

Table 2. ^{13}C NMR spectral data for compounds **1**–**4** (**1**,**2** and **4**; 125.65 MHz, **3**; 67.80 MHz, $CDCl_3$, residual $CHCl_3$ used as int. ref. $\delta = 77.00$).

C	**1**	**2**	**3**	**4**
1	59.01 (CH_2)	58.66 (CH_2)	59.32 (CH_2)	30.17 (Me)
2	124.49 (CH)	124.01 (CH)	123.54 (CH)	209.31 (C)
3	139.92 (C)	140.70 (C)	139.52 (C)	43.64 (CH_2)
4	31.92 (CH_2)	32.33 (CH_2)	39.55 (CH_2)	22.05 (CH_2)
5	26.31 (CH_2)	27.02 (CH_2)	26.26 (CH_2)	124.36 (CH)
6	124.44 (CH)	125.37 (CH)	125.41 (CH)	136.00 (C)
7	136.14 (C)	135.63 (C)	135.13 (C)	29.32* (CH_2)
8	32.21 (CH_2)	29.89* (CH_2)	29.60* (CH_2)	28.45* (CH_2)
9	26.61 (CH_2)	28.73* (CH_2)	28.81* (CH_2)	77.67 (CH)
10	124.17 (CH)	78.13 (CH)	78.33 (CH)	72.91 (C)
11	131.66 (C)	73.02 (C)	73.04 (C)	26.36 (Me)
12	25.69 (Me)	26.34 (Me)	26.43 (Me)	23.06† (Me)
13	17.62 (Me)	23.79† (Me)	23.25† (Me)	23.30† (Me)
14	23.31 (Me)	23.22† (Me)	23.21† (Me)	
15	23.44 (Me)	23.22† (Me)	16.48 (Me)	

Chemical shifts in ppm; multiplisities were determined by DEPT pulse sequence.
*,† Values are interchangeable within each column.



Scheme. 1. Possible pathways for the metabolism of **1** by *Glomerella cingulata*.

Preculture of Glomerella cingulata

G. cingulata was precultured by the same procedure described in previous papers [1, 2].

Time-course experiment

Precultured *G. cingulata* was transferred into a 200 ml Erlenmeyer flask containing 100 ml of medium, and stirred for 3 days. After growth of the fungus, compound **1** (50 mg) was added to the medium and the cultivation continued for 10 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 analyzed by TLC, GC and GC-MS. The relative concns of substrate (**1**) and metabolites were determined on the basis of GC peak area (Fig. 1).

Large-scale biotransformation of **1**

Precultured *G. cingulata* was transferred into a 3 l stirred fermentor containing 1 l of medium. Cultivation was carried out at 27° with stirring for 3 days and under aeration. After the growth of *G. cingulata*, **1** (500 mg) was added to the medium and then cultivation continued for 4 more days.

Isolation of metabolites

After the fermentation, the culture medium and mycelia were sepd by filtration. The medium was satd with NaCl and extracted with CH_2Cl_2. The mycelia were also extracted with CH_2Cl_2. The CH_2Cl_2 extracts were combined and the solvent was evapd to yield a crude extract (390 mg). The extract was sepd into the neutral (312 mg) and the acidic parts (17 mg) in the usual manner. The neutral part was subjected to CC on silica gel-60 with a hexane-Me_2Co stepwise from 19:1 to 1:1. The metabolites **2** (60 mg), **3** (28 mg) and **4** (16 mg) were isolated.

Compound **2**

Oil; $[\alpha]_{20}^{D}+3.3$ (CH_3Cl; *c* 0.65); FABMS (positive ion) *m/z* 257 $[MH]^+$; EI-MS *m/z* (rel. int.): 238 $[M\text{-}H_2O]^+$ (0.03), 220 $[M\text{-}2H_2O]^+$ (0.4), 205 $[M-2H_2O\text{-}CH_3]^+$ (0.8), 143 (8), 135 (10), 121 (12), 107 (14), 93 (36), 81 (74), 71 (63), 67 (41), 59 (100), 55 (38), 43 (99); IR ν_{max} cm^{-1}: 3374, 2964, 1669, 1448, 1378, 1224, 1164, 1079, 997; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Compound **3**

Oil; $[\alpha]_{20}^{D}+0.7$ (CH_3Cl; *c* 0.2); FABMS (positive ion) *m/z* 257 $[MH]^+$; EI-MS *m/z* (rel. int.): 238 $[M\text{-}H_2O]^+$ (0.01), 220 $[M\text{-}2H_2O]^+$ (0.1), 205 $[M\text{-}2H_2O\text{-}CH_3]^+$ (0.5), 143 (22), 135 (9), 121 (11), 107 (12), 93 (33), 81 (74), 71 (64), 67 (40), 59 (100), 55 (35), 43 (93); IR ν_{max} cm^{-1}: 3381, 2972, 1669, 1447, 1380, 1228, 1163, 1079, 999; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Compound **4**

Oil; EI-MS *m/z* (rel. int.): 210 $[M\text{-}H_2O]^+$ (0.4), 195 $[M\text{-}H_2O\text{-}CH_3]^+$ (0.3), 192 $[M\text{-}2H_2O]^+$ (0.8), 109 (18), 93 (23), 81 (16), 71 (26), 67 (15), 59 (54), 43 (100); IR ν_{max} cm^{-1}: 3424, 2970, 1709, 1376, 1163, 1078, 760; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Biotransformation of **2** *and* **3** *for 36 hours*

Precultured *G. cingulata* was transferred into a 50 ml Erlenmeyer flask containing 10 ml of medium, and stirred for 3 days. After the growth of the fungus, compound **2** and **3** (*ca* 5 mg) was added to the medium and the cultivation continued for 36 more hours. After the fermentation, the culture medium was satd with NaCl, extracted with EtOAc and the solvent then evapd. The crude extract was analyzed by GC and GC-MS.

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