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BIOTRANSFORMATION OF THE SESQUITERPENOID β-SELINENE USING THE PLANT PATHOGENIC FUNGUS *GLOMERELLA CINGULATA* AS A BIOCATALYST

MITSUO MIYAZAWA,* YOSHIHIRO HONJO and HIROMU KAMEOKA

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

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Key Word Index—*Glomerella cingulata*; plant pathogenic fungus; biotransformation; biocatalyst; sesquiterpenoid; β-selinene; (1*S*,6*S*,9*S*,10*R*,11*RS*)-1,11,13-trihydroxy-β-selinene.

Abstract—The biotransformation of β-selinene was investigated using the plant pathogenic fungus *Glomerella cingulata* as a biocatalyst. β-Selinene was oxidized at the double bond of the isopropenyl group and at the C-1 position regioselectively to (1*S*,6*S*,9*S*,10*R*,11*RS*)-1,11,13-trihydroxy-β-selinene. The structures of the metabolic products have been elucidated on the basis of their spectral data. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

We have been studying the biotransformation of terpenoids using a plant pathogenic fungus, *Glomerella cingulata*, as a biocatalyst. In our previous papers, some cyclic sesquiterpenoids {(−)-globulol [1], (−)-α-bisabolol [2], (+)-cedrol [3], (+)-aromadendrene [4] and (−)-alloaromadendrene [4]} were transformed into novel terpenes via stereoselective oxidation by *G. cingulata*. In this report, we describe the oxidation of (+)-aromadendrene and (−)-alloaromadendrene (sesquiterpene hydrocarbon) at the double bonded exomethylene and one of the methyl groups to form a triol. We were interested in stereospecific oxidation of cyclic sesquiterpene hydrocarbons by *G. cingulata*. β-Selinene (**1**) is a sesquiterpene hydrocarbon with a naphthalene skeleton and is a component of celery oil (*ca* 4%) [5]. There is a report that the autoxidation product of **1** has antimalarial activity [6]. However, the biotransformation of **1** has not been reported previously. Therefore, as part of our continuing programme, we investigated the biotransformation of **1** by *G. cingulata*. This paper describes the biotransformation of **1** into (1*S*,6*S*,9*S*,10*R*,11*RS*)-1,11,13-trihydroxy-β-selinene by *G. cingulata*.

RESULTS AND DISCUSSION

To investigate the time course of the biotransformation of **1** by *G. cingulata*, a small amount of **1** was incubated with *G. cingulata* for eight days. Two major products (**2** and **3**) and some minor products were detected by TLC and GC analysis. These products were not detected by TLC or GC analysis of the culture of *G. cingulata* to which substrate was not fed, and the mixture of **1** and culture medium was incubated under static conditions for eight days. From the above result, it was demonstrated that *G. cingulata* transformed **1** into **2** and **3** and some minor products. The time-course of concentration change of **1**–**3** was monitored by TLC and quantitatively measured by a GC method (Fig. 1). The starting substrate **1** was mainly transformed into **2** and **3**, and *ca* 50% of **1** was consumed after seven days. The major metabolites, **2** and **3**, respectively, accounted for *ca* 25% and *ca* 15% of recovered materials after seven days. To isolate these metabolites, a large scale incubation of **1** using *G. cingulata* was carried out for seven days. After the biotransformation, the culture was extracted as described in the Experimental, and metabolites **2** and **3** were isolated from the methylene dichloride extract. The structures of **2** and **3** were determined by spectral data.

Glomerella cingulata oxidized **1** at the double bond and hydroxylated at one of the methylenes on the naphthalene ring to form the triols **2** and **3**. The ^{1}H NMR spectrum of **2** displayed no signals for olefinic protons of an isopropenyl group, but there was the appearance of two signals at δ 3.34 (1H) and 3.44 (2H) due to an alcohol. The metabolite **2** was acetylated to yield triacetate **4** (pyridine–acetic anhydride). The ^{1}H NMR spectrum of **4** displayed three signals of methyl protons at δ 2.01, 2.04 and 2.08 due to the acetyl groups. In the ^{13}C NMR spectral data for **2** and **4** (Table 1), hydroxyl groups were revealed at C-1, C-11 and C-13 as primary, tertiary and secondary alcohols,

*Author to whom correspondence should be addressed.

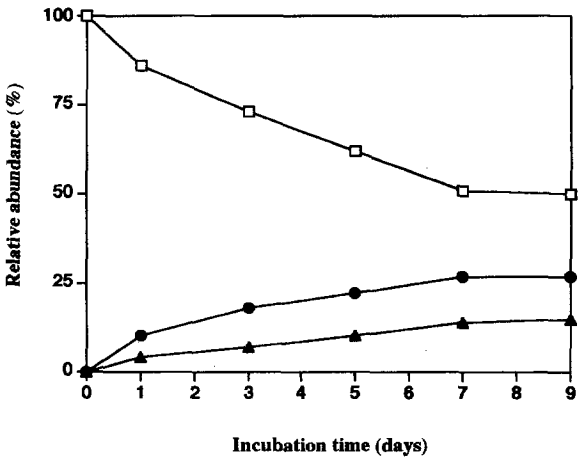
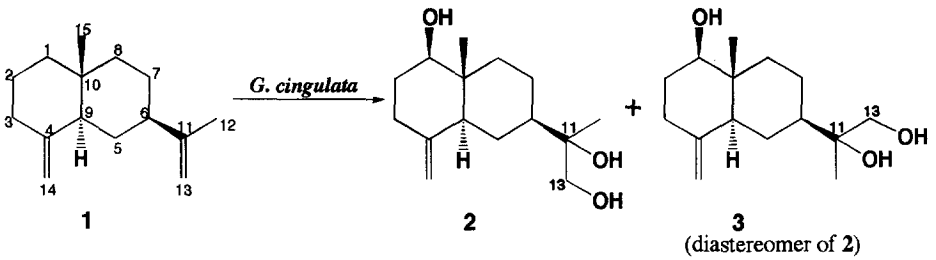


Fig. 1. Time course for the biotransformation of **1** by *G. cingulata*: □, β-selinene (**1**); ●, (1*S*)-1,11,13-trihydroxy-β-selinine (**2**); ▲, (1*S*)-1,11,13-trihydroxy-β-selinine (**3**).



respectively. Thus, the metabolite **2** was identified as 1,11,13-trihydroxy-β-selinene. To determine the absolute configuration of the secondary alcohol at C-1, compound **2** was converted into (*S*)- and (*R*)-MTPA esters of the acetonide. As a result of an adaptation of Mosher's method [7], C-1 was shown to have the

Table 1. ^{13}C NMR spectral data for compounds **1–5** (recorded at 67.80 MHz, residual $CHCl_3$ used as int. reference, $\delta = 77.00$)

C	1	2	3	4	5
1	41.95 (CH_2)[a]	80.03 (CH)	80.02 (CH)	84.34 (CH)	84.32 (CH)
2	23.47 (CH_2)	32.41 (CH_2)[c]	32.42 (CH_2)[d]	31.44 (CH_2)[e]	29.68 (CH_2)[g]
3	36.89 (CH_2)	35.45 (CH_2)[c]	35.44 (CH_2)[d]	34.11 (CH_2)[e]	33.75 (CH_2)[g]
4	150.98 (C)[b]	150.81 (C)	150.78 (C)	147.97 (C)	147.88 (C)
5	29.49 (CH_2)	24.68 (CH_2)	25.80 (CH_2)	24.33 (CH_2)	24.03 (CH_2)
6	45.84 (CH)	45.17 (CH)	45.27 (CH)	42.53 (CH)	42.59 (CH)
7	26.79 (CH_2)	23.36 (CH_2)	22.25 (CH_2)	23.16 (CH_2)	27.94 (CH_2)
8	41.18 (CH_2)[a]	38.35 (CH_2)[c]	38.31 (CH_2)[d]	36.71 (CH_2)[e]	36.30 (CH_2)[g]
9	49.87 (CH)	48.93 (CH)	48.83 (CH)	47.38 (CH)	47.44 (CH)
10	35.95 (C)	41.35 (C)	41.37 (C)	39.21 (C)	39.23 (C)
11	150.79 (C)[b]	75.45 (C)	75.39 (C)	80.39 (C)	80.42 (C)
12	21.01 (Me)	20.79 (Me)	21.11 (Me)	22.17 (Me)[f]	22.17 (Me)[h]
13	108.14 (CH_2)	69.12 (CH_2)	68.99 (CH_2)	65.47 (CH_2)	65.74 (CH_2)
14	105.35 (CH_2)	106.95 (CH_2)	106.87 (CH_2)	107.33 (CH_2)	107.38 (CH_2)
15	16.33 (Me)	10.72 (Me)	10.70 (Me)	11.19 (Me)	11.21 (Me)
COMe				170.26 (C)	170.23 (C)
				170.61 (C)	170.67 (C)
				170.73 (C)	170.76 (C)
$COCH_3$				18.38 (Me)[f]	18.79 (Me)[h]
				20.80 (Me)[f]	20.87 (Me)[h]
				21.14 (Me)[f]	21.20 (Me)[h]

Chemical shifts in ppm; multiplicities were determined by the DEPT pulse sequence.
[a–h] The assignments for these signals within the same column may be interchanged.

S-configuration. Compound **2** was the (1*S*,6*S*,9*S*, 10*R*,11*RS*)-form.

The ^{1}H NMR spectrum of **3** displayed no signals for olefinic protons due to an isopropenyl group, but three signals appeared at δ 3.34 (1H), 3.44 (1H) and 3.46 (1H) due to the alcohol. The metabolite **3** was acetylated to yield acetate **5** (pyridine–acetic anhydride). The spectral data for **3** and **5** were compared with those for **2** and **4**, respectively. As a result, the metabolite **3** was demonstrated to have same basic structure as **2**, i.e. 1,11,13-trihydroxy-β-selinene. In the ^{1}H NMR spectrum the chemical shift and coupling constant of H-1 agreed with those of **2**, which showed **3** was the (1*S*,6*S*,9*S*,10*R*,11*RS*)-form. In the ^{1}H NMR spectrum, the chemical shift and coupling value of the methylene protons at H-13 and H-13′ of **3** were not the same as those of **2**. Therefore, the metabolite **3** was identified as the diastereomer of 2.

The above results demonstrate that *G. cingulata* oxidized **1** at the double bond and hydroxylated at one of the methylene groups on the naphthalene ring to form the triols **2** and **3**. With regard to the oxidation of the double bond of (+)-aromadendrene [4] and (−)-alloaromadendrene [4], the metabolites were mainly oxidized at the double bond of the exomethylene group. However, in the biotransformation of **1** by *G. cingulata*, the oxidized double bond was not at the exomethylene, but at the isopropenyl group. This difference arises from a difference in their skeletons. The C-1 position of **1** was hydroxylated to yield the (1*S*)-alcohol stereoselectively by *G. cingulata* while the oxidation of the 11(13)-double bond did not proceed stereoselectively.

EXPERIMENTAL

Compound **1** was prepd by silica gel CC and vacuum distillation of celery oil (Yamamoto Perfumery Co.).

Preculture of G. cingulata. Spores of *G. cingulata* were inoculated on to a plate containing 15 ml of the following medium: 1.5% sucrose, 1.5% glucose, 0.5% polypeptone, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl, 0.1% K_2HPO_4 and 0.001% $FeSO_4 \cdot 7H_2O$. The culture was incubated at 27° under static conditions for 3 days.

Addition of β-selinene (**1**). After the growth of *G. cingulata*, 2.4 g of **1** (20 mg per 15 ml medium) was added to the medium, and the plates were left at 27° under static conditions for 7 days.

Isolation of metabolites **2** *and* **3**. After fermentation, culture broth was filtered to remove the mycelial mat. The filtrate was salted out with NaCl and extracted continuously with CH_2Cl_2 for 60 hr. The mycelical mat was extracted with CH_2Cl_2. The CH_2Cl_2 extracts were mixed and dried over Na_2SO_4 and the solvent was evapd to yield a crude extract (2.7 g). The extract was sepd into neutral (2.51 g) and acidic parts (178 mg) in the usual manner. The neutral part was subjected to CC on silica gel with a $CHCl_3$–MeOH gradient. In the $CHCl_3$ fr., unused **1** (1.19 g) was recovered. Metabolite **2** (233 mg) was isolated by recrystallization of the $CHCl_3$–MeOH (9:1) fr. The mother liquor was acetylated and chromatographed with a hexane–EtOAc gradient and the acetates of **2** and **3** were isolated. The acetates were deacetylated to yield **2** (33 mg) and **3** (146 mg). No metabolite in the acidic fr. was identified by GC-MS analysis.

Compound **2**. Crystals; mp: 141°; $[\alpha]_D^{20}$ +25.8° ($CHCl_3$; *c* 1.0). EIMS *m/z* (rel. int.): $[M - CH_2OH]^+$ 223(57), 205(37), 187(28), 177(5), 162(31), 147(29), 133(26), 119(41), 105(50), 91(47), 75(68), 57(41), 43(100). IR ν_{max} cm^{-1}: 3295, 2940, 2893, 1474, 1034. ^{1}H NMR (500.00 MHz, $CDCl_3$, TMS as int. standard): δ 0.66 (3H, *s*, H-15), 1.09 (3H, *s*, H-12), 3.34 (1H, *dd*, *J* = 4.5, 11.5 Hz, H-1), 3.44 (2H, *s*, H-13), 4.52 (1H, *br s*, H-14), 4.73 (1H, *br s*, H-14′). ^{13}C NMR data: Table 1.

Compound **3**. Crystals; mp: 137°; $[\alpha]_D^{20}$ +48.6° ($CHCl_3$; *c* 0.5). EIMS *m/z* (rel. int.): $[M - CH_2OH]^+$ 223(59), 205(37), 187(30), 177(4), 162(34), 147(32), 133(26), 119(40), 105(51), 91(39), 75(82), 57(42), 43(100); IR ν_{max} cm^{-1}: 3291, 2943, 2894, 1476, 1033. ^{1}H NMR (500.00 MHz, $CDCl_3$, TMS as int. standard): δ 0.66 (3H, *s*, H-15), 1.09 (3H, *s*, H-12), 3.34 (1H, *dd*, *J* = 4.5, 11.5 Hz, H-1), 3.44 (1H, *d*, *J* = 11.5 Hz, H-13), 3.46 (1H, *d*, *J* = 11.5 Hz, H-13′), 4.50 (1H, *br s*, H-14), 4.72 (1H, *br s*, H-14′). ^{13}C NMR data: Table 1.

Acetylation of compounds **2** *and* **3**. Pyridine (2 ml) was added to a soln of **2** (10 mg) in Ac_2O (30 ml), and the soln was refluxed for 5 hr. The product was treated with 1% HCl and 10% $NaHCO_3$ soln and extracted with CH_2Cl_2, and the solvent was evapd. The residue was sepd by silica gel CC, and the triacetate **4** (7 mg) was obtained. Similarly, **3** (6 mg) was acetylated to give the triacetate **5** (4 mg).

Compound **4**. Oil; EIMS *m/z* (rel. int.): $[M - HOAc]^+$ 320(1), 260(2), 200(9), 185(21), 171(7), 145(13), 117(19), 105(12), 91(10), 79(8), 55(6), 43(100); ^{1}H NMR (500.00 MHz, $CDCl_3$, TMS as int. standard): δ 0.76 (3H, *s*, H-15), 1.43 (3H, *s*, H-12), 2.00, 2.04, 2.08 (each 3H, *s*, CH_3CO), 4.68 (1H, *dd*, *J* = 4.5, 11.5 Hz, H-1), 4.35 (1H, *d*, *J* = 11.5 Hz, H-13), 4.52 (1H, *d*, *J* = 11.5 Hz, H-13′), 4.53 (1H, *br s*, H-14), 4.80 (1H, *br s*, H-14′). ^{13}C NMR data: Table 1.

Compound **5**. Oil; EIMS *m/z* (rel. int.): $[M - HOAc]^+$ 320(2), 260(2), 200(62), 185(38), 171(9), 145(12), 117(20), 105(15), 91(12), 79(9), 55(6), 43(100). ^{1}H NMR (500.00 MHz, $CDCl_3$, TMS as int. standard): δ 0.76 (3H, *s*, H-15), 1.44 (3H, *s*, H-12), 2.01, 2.04, 2.09 (each 3H, *s*, CH_3CO), 4.68 (1H, *dd*, *J* = 4.5, 11.5 Hz, H-1), 4.37 (1H, *d*, *J* = 11.5 Hz, H-13), 4.48 (1H, *d*, *J* = 11.5 Hz, H-13′), 4.50 (1H, *br s*, H-14), 4.76 (1H, *br s*, H-14′). ^{13}C NMR data: Table 1.

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