Journal of Molecular Catalysis B: Enzymatic 74 (2012) 6–8

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Stereoselective biocatalytic reduction of α-ionone by *Glomerella cingulata*

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ARTICLE INFO

Article history:
Received 27 April 2011
Received in revised form 30 July 2011
Accepted 30 July 2011
Available online 30 August 2011

Keywords:
Biotransformation
Glomerella cingulata
Stereoselective
α-Ionone
(−)-(6S,9R)-α-ionol

ABSTRACT

The biotransformation of (±)-α-ionone (**1**) by *Glomerella cingulata* was investigated. Compound **1** was transformed into two compounds (**2**,**3**). The ketone was reduced to α-ionol and the olefin was reduced to dihydrio-α-ionol, respectively. (−)-(6*S*,9*R*)- and (+)-(6*R*,9*S*)-α-ionol proceeded the corresponding allylic alcohols in high enantiomeric excess >99%; (−)-(*S*)-α-ionone is preferentially metabolized by hydroxylation in the ketone at C-9. Especially on the metabolic pathway, olefine reduction was via after ketone reduction.

1. Introduction

Terpenoids are known as not only raw materials for flavor and fragrance but also biologically active substances. A great majority of biologically active terpenoids are produced as plant secondary metabolites, and these terpenoids have been shown to have biological activity against plants, microorganisms, and insects. Various attempts have been made to search for new biologically active terpenoids. Biotransformation is one way to produce biologically active terpenoids.

We have studied the microbial transformation of terpenoids. In our previous papers, (−)-globulol, (+)-γ-ledrol [1], (±)-aromadendrene [2], (+)-γ-gurjunene [3], α-bulnesene [4], (+)-cycloisolongifolol [5] and (±)-bornylacetate [6] transformed into terpenes via stereoselective methods by *Glomerella cingulata*.

Ionone is an important intermediate in the metabolism of terpenoids, e.g., in carotenoid biosynthesis, and has been isolated from many sources. Compounds with a trimethylcyclohexane building block constitute essential aroma elements in many plant oils and thus have attracted the attention of the flavor and fragrance industry. In the past, biotransformation of α-ionone (**1**) used microbial of *Aspergillus nigeri* [7], *Rhodococcus rubber* [8], *Trichosporum cutaneum* [9], *Storeptomyces grisus* [10] and *Storeptomyces hygroscopicus* [10], and plant cell cultures of *Caragana chamlagu* [11] and *Nicotina tabacum* [12] were investigated.

The aim of this study was to study the stereoselective biotransformation of α-ionone by *G. cingulara.* Whole cells exploiting its oxygenase activity. The stereochemical consequences of the transformations are also discussed.

2. Results and discussion

α-ionone (**1**) was in the proportion of 1:1 to (6*S*) and (6*R*), chiral GC showed. In order to investigate the time course of the biotransformation of α-ionone (**1**) by *G. cingulata*, a small amount of compound **1** was incubated with *G. cingulata* for 10 days. Four metabolites were detected by TLC, GC, and GC–MS analyses (Figs. 1 and 2). The conversion rate of metabolites (−)-(6*S*,9*R*)-α-ionol (**2a**), (+)-(6*R*,9*S*)-α-ionol (**2b**), (−)-(6*S*,9*R*)-dihydro-α-ionol (**3a**) and (+)-(6*R*,9*S*)-dihydro-α-ionol (**3b**) was 35%, 28%, 18%, and 8%, respectively for 10 days.

In order to isolate those metabolites, a large-scale incubation of compound **1** with *G. cingulata* was carried out for 10 days. After the biotransformation, the culture was extracted as described in Section 3. The structure of metabolites were isolated from the EtOAc extract and determined by spectral data.

Compounds **2a** and **2b** were isolated for mixture and had a molecular formula $C_{13}H_{22}O$, as determined by mass spectrum and NMR data. The IR spectrum contained a hydroxyl band 3350 cm^{-1} and the specific rotation of mixture of compounds **2a** and **2b** were −6.29°. The ^{1}H and ^{13}C NMR spectra of compounds indicated the presence of an alcohol group at C-9 (Tables 1 and 2). These NMR data were found to agree with the previous paper [13,14]. The result allowed us to identify compounds as α-ionol [comparison to literature [15] (Figs. 2 and 3].

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doi:10.1016/j.molcatb.2011.07.021

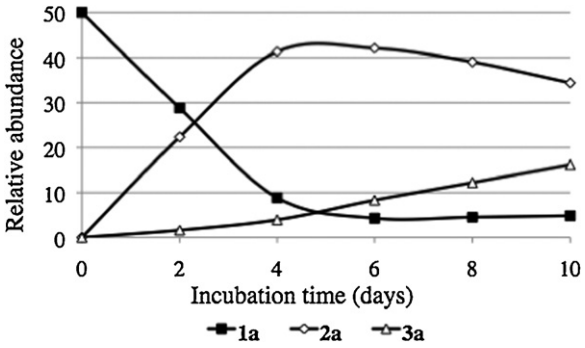

Fig. 1. Time course of the biotransformation of **1a** by *G. cingulata* (■) (−)-(6*R*)-α-ionone (**1a**); (◊) (−)-(6*S*,9*R*)-α-ionol (**2a**); (△) (−)-(6*S*,9*R*)-7,8-dihydro-α-ionol (**3a**).

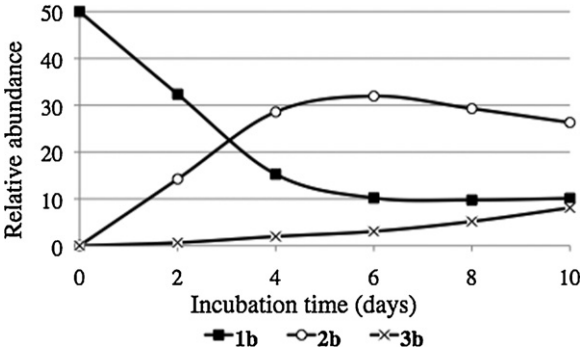

Fig. 2. Time course of the biotransformation of **1b** by *G. cingulata* (■) (+)-(6*R*)-α-ionone (**1b**): (○) (+)-(6R,9S)-α-ionol (**2b**); (×) (+)-(6*R*,9*S*)-7,8-dihydro-α-ionol (**3b**).

Table 1
^{1}H NMR spectral data of metabolite (*d* in ppm, *J* in Hz).

No	**2a** and **2b**	**3a** and **3b**
1		
2	1.42 (2H, *m*)	1.45 (2H, *m*)
3	1.62 (2H, *m*)	1.63 (2H, *m*)
4	5.32 (1H, *t*, *J* = 4.0)	5.29 (1H, *t*, *J* = 6.0)
5		
6	2.23 (1H, *d*, *J* = 6.2)	1.93 (1H, *d*, *J* = 7.0)
7	5.53 (1H, *dd*, *J* = 15.2, *J* = 6.2)	1.52 (1H, *dd*, *J* = 7.4, *J* = 7.0)
8	5.43 (1H, *dd*, *J* = 15.2, *J* = 7.4)	1.59 (1H, *dd*, *J* = 7.4, *J* = 5.9)
9	4.12(1H, *d*, *J* = 7.4)	1.18(1H, *d*, *J* = 5.9)
10	1.56 (3H, *s*)	1.29 (3H, *s*)
11	0.85 (3H, *s*)	0.87 (3H, *s*)
12	0.89 (3H, *s*)	0.92 (3H, *s*)
13	1.99 (3H, *s*)	1.82 (3H, *s*)

NMR spectra were recorded at 400 MHz (^{1}H) in $CDCl_3$ solution using tetramethylsilane (TMS) as internal standard.

Table 2
^{13}C NMR spectral data of metabolite.

No	**2a** and **2b**	**3a** and **3b**
1	31.0	34.0
2	29.6	31.0
3	22.3	22.2
4	120.7	121.8
5	133.9	135.7
6	53.9	49.1
7	131.3	19.4
8	135.8	44.9
9	68.9	68.5
10	23.6	23.5
11	26.7	28.3
12	26.7	28.3
13	26.7	21.5

NMR spectra were recorded at 100 MHz (^{13}C) in $CDCl_3$ solution using tetramethylsilane (TMS) as internal standard.

Compounds **3a** and **3b** were isolated for mixture and had a molecular formula $C_{13}H_{24}O$, as determined by mass spectrum and NMR data. The IR spectrum contained a hydroxyl band 3350 cm^{-1} and the specific rotation of mixture of compounds **3a** and **3b** was −10.56°. The ^{1}H and ^{13}C NMR spectra of compounds indicated the presence of an alcohol group at C-9 and disappearance of a double bond at C-7/C-8 (Table 1). These NMR data were found to agree with the previous paper [16,17]. The result allowed us to identify compounds as 7,8-dihydro-α-ionol [comparison to literature [16] (Figs. 1 and 2)].

As a result, compound **1** was transformed to **2a** and **2b**, which contain one pathway (Fig. 1). Compounds **2a** and **2b** were transformed into **3a** and **3b**.

In conclusion, there were three stereoselective compounds. The first, (−)-(6*S*,9*R*)- and (−)-(6*R*,9*S*)-**2** proceeded the corresponding allylic alcohols in high enantiomeric excess >99%, which leads to the conclusion that recognize stereochemistry at C6 of γ-position in α,γ-unsaturated ketones and reduced ketone at C9. The second, (−)-(*S*)-α-ionone is preferentially metabolized by hydroxylation in the ketone at C-9, this reaction dominantly produce (−)-(6*S*,9*R*)-α-ionol (**2a**) and (−)-(6*S*,9*R*)-7,8-dyhidro-α-ionol (**3a**) (Figs. 1 and 2). Finally, this conversion route was unique. This pathway became reduced ketones first, and then, olefin was reduced by *G. cingulata*. This conversion route with stereoselective is not precedent for this sort of thing in biotransformation by microorganism as a biocatalyst. Biotransformation of *G. cingulata* did not report reduction of α,γ-unsaturated ketones by *G. cingulata*. We reported to be marked by the high ability to reduction of ketones by enzymatic of *G. cingulata* [18–21]. So, this conversion route reduced ketones of α,γ-unsaturated ketones first, and these enzymatic reactions were catalyzed by *G. cingulata*.

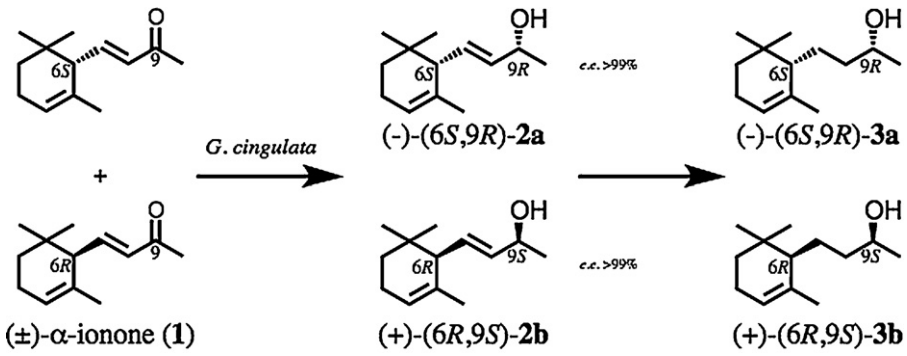

Fig. 3. Biotransformation of (±)-α-ionone by *G. cingulata*.

3. Experimental

JMS-700 TKM. Nuclear magnetic resonance (NMR) spectra were recorded at 500 MHz for 1H and 125 MHz for 13C on a JEOL ECA-500 spectrometer. IR spectra were determined with a JASCO FT/IR-470 plus Fourier transform infrared spectrometer. Thin-layer chromatography (TLC) was performed on precoated plates (Silica gel 60 F254, 0.25 mm, Merck). The mobile phase was hexane–EtOAc. Compounds were visualized by spraying plates with 0.5% vanillin in 96% H_2SO_4 followed by brief heating.

3.1. Biotransformations

3.1.1. Preculture of G. cingulata

Preculture of *G. cingulata*. Spores of *G. cingulata* NBRC 5952 (NITE Biological Resource Center, Japan), which had been preserved on potato dextrose agar (PDA) at 4 °C, were inoculated into 100 mL of sterilized culture medium (1.5% saccharose, 1.5% glucose, 0.5% polypeptone, 0.05% $MgSO_4{\cdot}7H_2O$, 0.05% KCl, 0.1% K_2HPO_4, and 0.001% $FeSO_4{\cdot}7H_2O$ in distilled H_2O) in a 300 mL shaking flask, and the flask was shaken (reciprocating shaker, 120 rpm) at 27 °C for 7 days.

3.1.2. Time-course experiment

Precultured *G. cingulata* (2 mL) was transferred into a 300 mL Erlenmeyer flask containing 100 mL of medium and stirred (ca. 120 rpm) for 5 days. After the growth of *G. cingulata*, 30 mg of **1** in 0.5 mL of dimethyl sulfoxide (DMSO) was added to the medium and the organism cultivated 10 days. Every other day, 5 mL of each culture medium was removed, saturated with NaCl, extracted with EtOAc and the solvent then evaporated. The crude extracts were analyzed by TLC, GC, and GC–MS. The relative concerns of substrate and their metabolites were determined on the basis of GC peak area respectively.

3.2. Substrates

(±)-α-Ionone (**1**) was purchased from Fluka Chemical (Tokyo, Japan).

3.3. Isolation of metabolites

Precultured *G. cingulata* was transferred into four 1000 mL Erlenmeyer flask containing 500 mL of medium. Cultivation was carried out at 27 °C with stirring (ca. 120 rpm) for 5 days. After the growth of *G. cingulata*, 150 mg of **1** (0.3 mg/mL) in 2.5 mL of DMSO was added to the medium and the organism cultivated 10 days each other. At the end of the incubation period, the culture medium was collected, saturated with NaCl and extracted with EtOAc. The mycelia were also collected and extracted with EtOAc. The EtOAc extracts were combined and the solvent was removed under reduced pressure. The extract was chromatographed on silica gel using *n*-hexane–EtOAc and the recovery substrate and metabolites mixture of **2a** and **2b** (36 mg) and mixture of **3a** and **3b** (13 mg) were isolated and purified.

3.4. GC analyses

3.4.1. Non-chiral methods

Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector, a HP-5 capillary column (30 m length, 0.25 mm i.d.), and a split injection of 20:1 were used. Helium at a flow rate of 0.6 mL/min was used as a carrier gas. The oven temperature was programmed from 80 to 280 °C at 4 °C/min. The injector was 270 °C. The detector temperature was 280 °C. The peak area was integrated with a Hewlett-Packard HP3396 Series2 injector. Retention time: mixture **2a** and **2b** (16.3 min); mixture **3a** and **3b** (22.8 min).

3.4.2. Chiral methods

Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector, a Cyclodextrine-β-236M-19 (50 m × 0.25 mm i.d.), and a split injection of 20:1 were used. Helium at a flow rate of 0.6 mL/min was used as a carrier gas. The oven temperature was programmed from 80 to 220 °C at 10 °C/min. The injector was 210 °C. The detector temperature was 220 °C. The peak area was integrated with a Hewlett-Packard HP3396 Series2 injector. Retention time: (6*S*,9*R*)-**2a**, (18.3 min); (6*R*,9*S*)-**2b**, (19.1 min); (6*S*,9*R*)-**3a**, (21.2 min); (6*R*,9*S*)-**3b**, (22.0 min).

3.5. Determination absolute configuration

The assignment for **2a** and **2b** was performed by comparison of the elution the order on chiral GC [19].

3.6. (6SR,9RS)-α-ionol (*2a* and *2b*)

Colorless oil; $[\alpha]_D^{25}$ −6.29° (c 1.0, $CHCl_3$); IR ν_{max} (film) 3350 cm^{-1}; ^1H and ^{13}C NMR data (Tables 1 and 2); EIMS *m/z* 194 [M^+] (2), 161 (1), 138 (34), 123 (32), 95 (100), 91 (11), 79 (13), 55 (12), 43 (42).

3.7. (6SR,9RS)-7,8-dihydro-α-ionol (*3a* and *3b*)

Colorless oil; $[\alpha]_D^{25}$ −7.28° (c 1.0, $CHCl_3$); IR ν_{max} (film) 3350 cm^{-1}; ^1H and ^{13}C NMR data (Tables 1 and 2); EIMS *m/z* 196 [M^+] (10), 181 (3), 163 (13), 136 (14), 123 (32), 107 (40), 93 (100), 31 (38), 55(21), 41 (28).

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