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Highly Selective and Asymmetric Reductive Biotransformation of α-Ionone by *Epicoccum purpurascens*

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Abstract: The biotransformation of terpenoid C13 norisoprenoid (±)-α-ionone (1) using the plant pathogenic fungus *Epicoccum purpurascens* as a biocatalyst was investigated for the production of useful novel organic compounds. There are no reported biotransformations using *E. purpurascens*. The biotransformation of compound 1 via reduction of the C-9 ketone position yielded α-ionol (2) as the major metabolic product. Reduction of the racemic α-ionone [(−)-(6*S*)- and (+)-(6*R*)-] resulted in the exclusive formation of the two enantiomers (−)-(6*S*,9*R*)- and (+)-(6*R*,9*S*)-α-ionol (2). Thus, the enzymatic reduction of α-ionone by *E. purpurascens* proceeds with high asymmetry.

Key words: Biotransformation; *Epicoccum purpurascens*; Stereoselective; Asymmetry reduction; (±)-α-ionone; (6*SR*,9*RS*)-α-ionol.

1 Introduction

Ionones and their derivatives are important intermediates in the metabolism of terpenoids, e.g., in carotenoid biosynthesis, and have been isolated from many sources[1]. Compounds possessing trimethylcyclohexane building blocks constitute essential aromatic elements in many plant oils. This norisoprenoid is widely found in various quantities in essential oils, vegetables, fruits, tea, and tobacco. α-Ionones are found in many plants[2−5] and often constitute flavors (e.g., berry flavors such as raspberry) and are used in the fragrance industry (violet, floral, woody, and rose notes)[6−9]; in addition, α-ionones also exhibit bioactivity[10−12]. Furthermore, ionone derivatives such as 3-hydroxy-α-ionones could prove valuable intermediates for the chemoenzymatic synthesis of carotenoids, e.g., astaxanthin and zeaxanthin[13]. Uncultured fungi are derived primarily from sediments[14−16], wood[17], and algae[18,19]. Thus, biotransformation can be defined as the use of biological systems to produce chemical changes in compounds that are not their natural substrates. Biotransformation is more advantageous as compared to the corresponding chemical transformations. Biotransformation conditions are generally mild and usually do not require the protection of other functional groups. In many cases, biotransformations are stereoselective, allowing the production of enantiomerically pure compounds. We have studied the biotransformation of **1** by the plant pathogenic fungus *Glomerella cingulata*. In our previous studies, compound **1** was transformed to α-ionol and dihydro α-ionol[20]. Recently, it has been reported that *Epicoccum purpurascens* shows promise for biodiesel production[21] and can be isolated from diseased berries[22]. However, there has been no report on the biotransformation of terpenoids by *E. purpurascens* to date[23]. In this study, the potential of cultured cells of *E. purpurascens* was evaluated in the biotransformation of **1**.

2 Experimental

2.1 General

The substrate used for the biotransformation experiments was (±)-α-ionone (**1**) (Fluka Chemie). Also, NMR: 500 MHz (^{1}H), 125 MHz (^{13}C), $CDCl_3$ with TMS as the internal standard, GC: Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector, a cyclodextrin-β-236M-19 (50 m × 0.25 mm i.d.) column, and a split injection of 20:1 were used. Helium was used as the carrier gas at a flow rate of 0.6 mL/min. The oven temperature was programmed to increase from 80 to 220℃ at a rate of 2℃/min. The injector was set at 210℃ and the detector temperature was 220℃[24]. The peak area was inte-

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grated using a Hewlett-Packard HP3396 Series 2 integrator. GC-MS was carried out on a Hewlett-Packard 5890A gas chromatograph equipped with a split injector HP-5MS capillary column (30 m × 0.25 mm i.d.), which was directly coupled to a Hewlett-Packard 5972A mass spectrometer. The same temperature program as described above for GC was used. Helium was used as the carrier gas with a flow rate of 1 mL/min. The temperature of the ion source was 280℃, and the electron energy was 70 eV. The electron impact (EI) mode was used. IR spectra were obtained with a Perkin-Elmer 1760X spectrometer. $CHCl_3$ was used as a solvent. TLC was performed using precoated silica gel 60 F254 plates (layer thickness 0.25 mm, Merck). Compounds were visualized by spraying the plates with 0.5% vanillin in 96% H_2SO_4 followed by brief heating; CC: silica gel developed with hexane-EtOAc.

2.2 Preculture of *E. purpurascens*

In the preculture of *E. purpurascens*, spores of *E. purpurascens* NBRC 30392 (NITE Biological Resource Center, Japan), which had been preserved on potato dextrose agar (PDA) at 4℃, 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, 0.001% $FeSO_4 \cdot 7H_2O$, and distilled water; pH 7.2) in a 300-mL shaking flask, and the flask was shaken (reciprocating shaker, 120 rpm) at 27℃ for 7 d.

2.3 Time-course experiment

Precultured *E. purpurascens* (2 mL) was transferred into a 300-mL Erlenmeyer flask containing 100 mL of culture medium and stirred (ca. 120 rpm) for 5 d. After the growth of *E. purpurascens*, **1** (0.156 mmol) in 0.5 mL of dimethyl sulfoxide (DMSO) was added to the medium and the culture was continued for 10 d. Every other day, 5 mL of culture medium was removed, salted out with NaCl, and then extracted with EtOAc. The extract was analyzed by GC. The ratios of the substrate to the metabolic products were determined via TLC on the basis of the peak areas obtained from GC-MS (Fig. 1).

2.4 Biotransformation of (±)-α-ionone (1) for 10 d

Precultured *E. purpurascens* was transferred into four 1000-mL Erlenmeyer flasks containing 500 mL medium. Cultivation was carried out at 27℃ with stirring (ca. 120 rpm) for 5 d. After the growth of *E. purpurascens*, 150 mg of **1** in 2.5 mL of DMSO was added to the medium and the organism was cultivated for 10 d.

2.5 Isolation of metabolite

After biotransformation for 10 d, the culture medium and mycelia were separated by filtration. The medium was salted out with NaCl and extracted with EtOAc. The mycelia were also extracted with EtOAc. The EtOAc extracts were mixed and dried over Na_2SO_4, and the solvent was evaporated to yield the crude extract (1.02 g). The extract was chromatographed on silica-200 columns with hexane-EtOAc, and untransformed **1** (360 mg) was recovered.

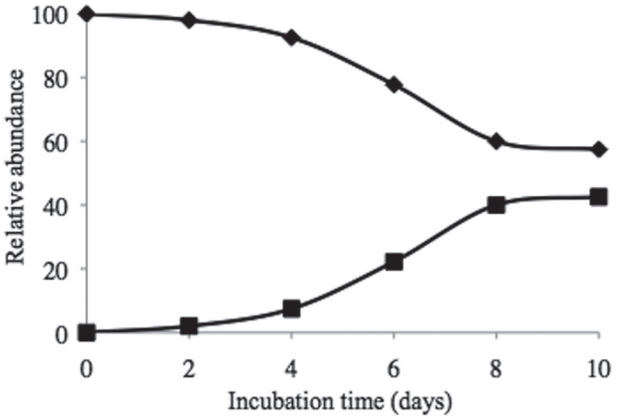

Fig. 1 Time course of the biotransformation of **1** by *E. purpurascens*: (◆) α-ionone (**1**); (■) α-ionol (**2**).

2.6 Synthesis of four stereoisomers of α-ionol (2) from α-ionone (1)

A mixture of four stereoisomers of α-ionol (**2**) was obtained upon $LiAlH_4$ reduction of a racemic mixture of (±)-α-ionone (**1**) in dry tetrahydrofuran (THF) in an ice bath. Under suitable conditions, four baseline-separated peaks (Fig. 3a) were obtained for the four stereoisomers of **2**.

2.7 (6*SR*,9*RS*)-α-ionol (2a and 2b)

Colorless oil, $[\alpha]^{25}_{D}$ -19.52° (c 1.0, $CHCl_3$), IR: ν_{max} (film): 3350 cm^{-1}, ^{1}H-NMR ($CDCl_3$): δ 0.90 (3H, *s*, H-11), 0.94 (3H, *s*, H-12), 1.45 (2H, *m*, H-2), 1.61 (3H, *s*, H-10), 1.67 (2H, *m*, H-3), 2.04 (3H, *s*, H-13), 2.28 (1H, *d*, *J* = 6.2 Hz, H-6), 4.17 (1H, *d*, *J* = 7.4 Hz, H-9), 5.37 (1H, *t*, *J* = 4.0 Hz, H-4), 5.48 (1H, *dd*, *J* = 15.2 Hz, *J* = 6.2 Hz, H-7), 5.58 (1H, *dd*, *J* = 15.2 Hz, *J* = 7.4 Hz, H-8). ^{13}C-NMR ($CDCl_3$): δ 22.6 (C-6), 23.1 (C-13), 23.4 (C-10), 26.9, 27.1 (C-11, 12), 29.9 (C-2), 31.3 (C-1), 54.3 (C-6), 68.5 (C-9), 121.0 (C-4), 131.0 (C-7), 134.1 (C-5), 135.5 (C-8). EIMS *m/z* 194 [M^+] (2), 161 (1), 138 (34), 123 (32), 95 (100), 91 (11), 79 (13), 55 (12), 43 (42).

3 Results and discussion

Chiral gas chromatography (GC) demonstrated that (±)-α-ionone (**1**) was present as the (6*S*) and (6*R*) isomers in a 1:1 proportion. Time-course experiments were performed by incubating a small amount of **1** with *E. purpurascens* for 10 d. A major metabolite was detected by means of

TLC, GC, and GC-MS analyses. This product was not detected in the TLC, GC, and GC-MS analyses of two controls containing either mycelia with the medium or the substrate dissolved in DMSO with the medium. No metabolic product was observed in the two controls. In addition, the biotransformation using *E. purpurascens* reproducibly yielded **2** under the culture conditions.

Thus, it is concluded that *E. purpurascens* transformed **1** to **2**. The time-course of the metabolites shown in Fig. 1 demonstrates that about 40% of **1** was metabolized. In order to isolate the metabolite, a large-scale incubation of **1** with *E. purpurascens* was carried out, and the culture was extracted as described in Section 2. Metabolites were isolated from the EtOAc extract and their structures were determined on the basis of spectral data. Compound **2** was isolated from the mixture, and its molecular formula was determined as $C_{13}H_{22}O$ by means of mass spectroscopy and NMR. A hydroxyl band was detected at 3350 cm^{-1} in the IR spectrum, and the ^{1}H- and ^{13}C-NMR spectra of the compound indicated the presence of an alcohol group at C-9. These NMR data were found to be in agreement with the previous report[24]. Based on the spectral analyses, the compound was identified as α-ionol (**2**) (Fig. 2). It was expected that the four diastereoisomers of the hydroxy product (Fig. 3a) would be obtained using racemic **1** as a starting material. The formation of compound **2** by biotransformation was determined from chiral GC analysis using the β-cyclodextrin-based column; notably, only two major product peaks (Fig. 3c) representing the two enantiomers, (−)-(6*S*,9*R*)-α-ionol (**2a**) and (+)-(6*R*,9*S*)-α-ionol (**2b**), were observed (Fig. 2). Furthermore, chiral GC shows two peaks (Fig. 3c) that merge into a single peak if an achiral phase is used. Thus, enzymatic hydroxylation of α-ionol (**2**) by the *E. purpurascens* strains proceeds with both high regio and stereoselectivity. The conversion ratios of the metabolites (−)-(6*S*,9*R*)-(**2a**) and (+)-(6*R*,9*S*)-(**2b**) were 50% and 23%, respectively. The specific rotation of the mixture of **2a** and **2b** was −19.52°. These results demonstrate that *E. purpurascens* effected the reduction of **1** to yield one major metabolite involving the reduction of the C-9 ketone position to **2**. Reduction of (−)-(6*S*,9*R*)- and (+)-(6*R*,9*S*)-**2** proceeded to generate the corresponding allylic alcohols via highly selective asymmetric reduction, which leads to the recognized stereochemistry at C-6 of the γ-position in α, β-unsaturated ketones and reduction of the ketone at C-9. The same metabolites, (−)-(6*S*,9*R*)- and (+)-(6*R*,9*S*)- **2**, were previously obtained in 6:4 ratio via biotransformation using *G. cingulata*[20]. However, the reductive biotransformation by *E. purpurascens* is characterized by higher selective asymmetry than that of *G. cingulata*, leading to an isomeric ratio of 7:3. Furthermore, *E. purpurascens* leads to the highly efficient transformation of a racemate into a single stereoisomeric product in (theoretically) 100% chemical yield and 100% enantiomeric excess, the specific merits and limitations of which were discussed. This conversion route reduced α, β-unsaturated ketones via enzymatic reactions catalyzed by *E. purpurascens*. Because the Eipcoccum species is suitable for screening specific human drug-metabolizing enzymes (CYP2C9)[25], this biocatalysis may be useful as a means for screening tests.

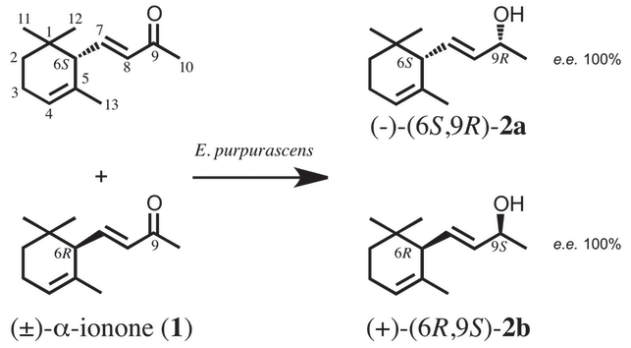

Fig. 2 Biotransformation of α-ionone (**1**) by *E. purpurascens*.

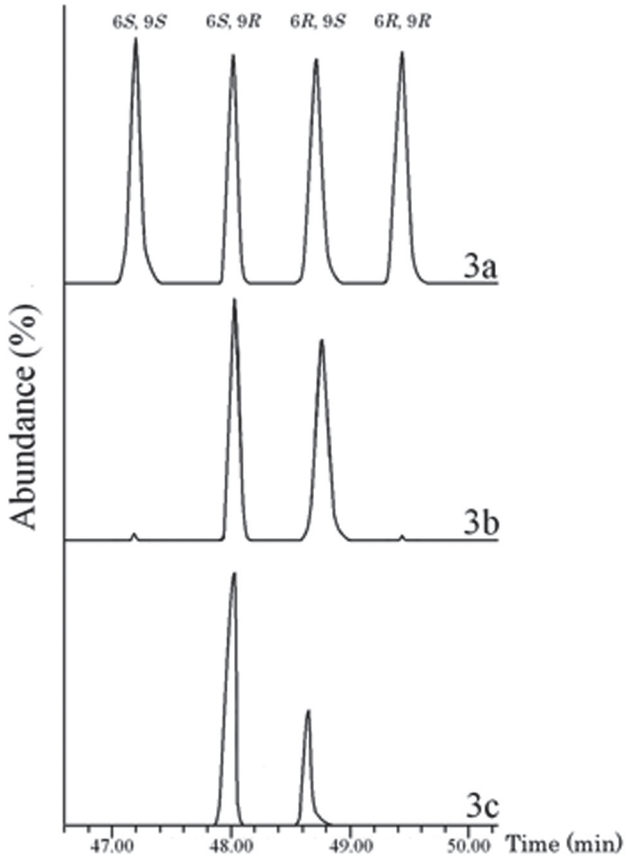

Fig. 3 Chiral GC peak of α-ionol: (3a) Synthesis four streoisomers α-ionol; (3b) Biotransformation by *G. cingulata*.; (3c) Biotransformation of *E. pururascens*.

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