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ENANTIOSELECTIVE ACCUMULATION OF (−)-PINORESINOL THROUGH *O*-DEMETHYLATION OF (±)-EUDESMIN BY *ASPERGILLUS NIGER*

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Key Word Index—*Aspergillus niger*; fungus; (±)-eudesumin; (−)-pinoresinol; *o*-demthylation; enantioselective accumulation.

Abstract—Microbial transformation of (±)-eudesmin by *Aspergillus niger* was investigated. Enantioselective accumulation of (−)-pinoresinol was shown through *O*-demethylation of (±)-eudesmin. This fungus *O*-demethylated both enantiomers of eudesmin, but the conversion rates for each enantiomer were clearly different. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Pinoresinol is a well known lignan which is distributed widely in the plant kingdom [1, 2] and its derivatives show a variety of biological activities [2, 3]. Naturally occurring pinoresinol usually has a (+)-configuration and has been isolated from more than 56 plant species (e.g. *Eucommia ulmoides* [4], *Magnolia fargesii* [5] and *Fraxinus japonica* [6]). However (−)-pinoresinol occurs only in eight plant species (e.g. *Xanthoxylum ailanthoides* [7]). Because of those enantioselective production of (+)-pinoresinol, there are many lignans which might be biosynthesized from (+)-pinoresinol in the plant kingdom, e.g. (+)-eudesmin [8, 9] and (+)-pinoresinol mono- and di-glycoside [4]. As part of our investigation on biotransformations of lignans, we previously reported the biotransformation of (+)-eudesmin by a fungus (*Aspergillus niger*) [10] and an insect (*Spodoptera litura*) [11]. As a result, we found that (+)-eudesmin was *O*-demethylated and transformed to (+)-pinoresinol by *A. niger* and to pinoresinol monomethyl ether glucoside by *S. litura*. We attempted to elucidate the metabolic pathway of (±)-eudesmin, (±)-pinoresinol monomethyl ether and (−)-eudesmin, a minor product in nature, in these organisms. In this report, we deal with the microbial transformation of (±)-eudesmin, (±)-pinoresinol monomethyl ether and (−)-eudesmin by *A. niger*; the enantioselective accumulation of (−)-pinoresinol is shown through *O*-demethylation of (±)-eudesmin.

RESULTS AND DISCUSSION

(±)-Eudesmin (**1**) was added onto the surface of mycelial mat and incubated at 28°, then extracted with ethyl acetate. NMR, IR and GC-mass spectral analysis supported the assumption that pinoresinol monomethyl ether (**2**) and pinoresinol (**3**) was accumulated through a *O*-demethylation process as reported previously [10]. The ratio of compound **1** and metabolites **2** and **3** varied with time (Fig. 1); the enantiomer excesses of each compound were measured constantly by chiral HPLC. As a result, (−)-pinoresinol was preferentially accumulated (ee 62%) after 72-hr incubation; unreacted (−)-eudesmin had ee 29.5%.

It was found that when compound **1** (170 mg) was incubated at 25° for 72 hr, compounds **2** (3 mg), **3** (24 mg) and unreacted compound **1** (48 mg) were obtained; compound **3** had $[\alpha]_D$ −57.6 (*c* 1.2) and an enantiomer excess of 100% by chiral HPLC analysis. Metabolite **2** had $[\alpha]_D$ −12.8 (*c* 0.15) and ee 39.2% and unreacted **1** showed $[\alpha]_D$ −1.79 (*c* 2.4) and ee 0.92% by chiral HPLC analysis.

In both cases, the amount of exhausted **1a** was the same or greater than that of **1b**, while **3b** accumulated with high enantiomer excess. These results suggest that *A. niger* metabolized both enantiomer of compound **1**; however, compound **3a** was metabolized

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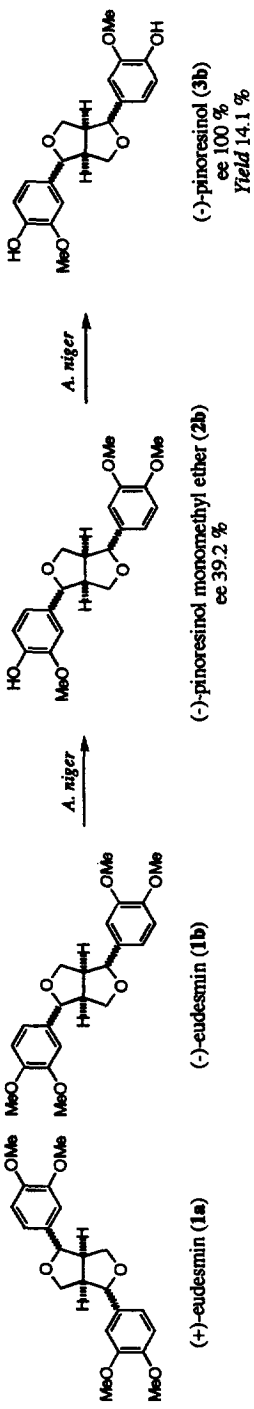

Scheme 1. Accumulation of (−)-pinoresinol (**3b**) by *O*-demethylation of (±)-eudesmin by *Aspergillus niger*.

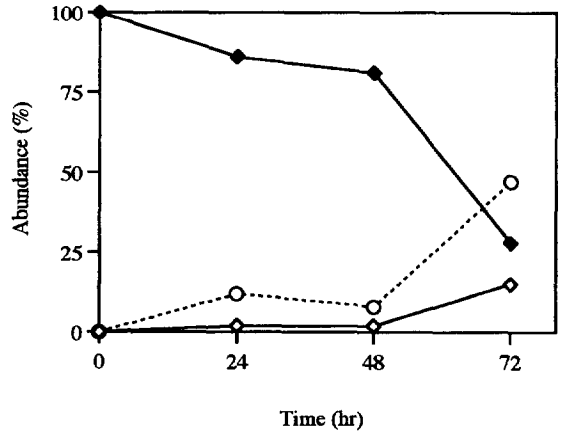

Fig. 1. Time-course of (±)-eudesmin (**1**, ◆) and metabolites **2** (◇) and **3** (○).

further and, consequently, **3b** was accumulated. We previously reported that compound **3a** was oxidized to 5′-hydroxypinoresinol [10]; however, in the above incubation, this compound was not detected by TLC and GC-mass spectrometry, and the amount of unreacted compound **1** was less than that of calculated values. Therefore, it is presumed that **3a** might be converted to smaller molecules by this fungus.

Biotransformation of (±)-pinoresinol monomethyl ether (**2**) by *A. niger* was also examined. The ratio between metabolites **2** and **3** varied with time (Fig. 2). After a 72-hr incubation at 25°C, compound **2** was converted to compound **3** (39%). Unreacted compound **2** gave a negative optical rotation and its enantiomer excess was 25.2%. Metabolite **3** also had the (−)-form and its enantiomer excess was 37.2%. These results also suggested that metabolite **3a** was further transformed to any other compound.

Biotransformation of (−)-eudesmin (**1b**) by *A. niger* was investigated in order to compare the conversion rate with that of **1a**, and of **1b** in (±)-eudesmin. The time-conversion rate relationships of **1a**, **1b** with their metabolites are shown in Figs 3 and 4. After a 48-hr incubation at 25°, **1b** was completely

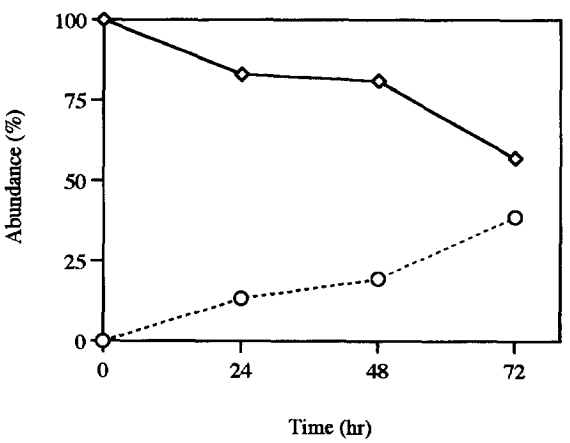

Fig. 2. Time-course of (±)-pinoresinol monomethyl ether (**2**, ◇) and metabolite **3** (○).

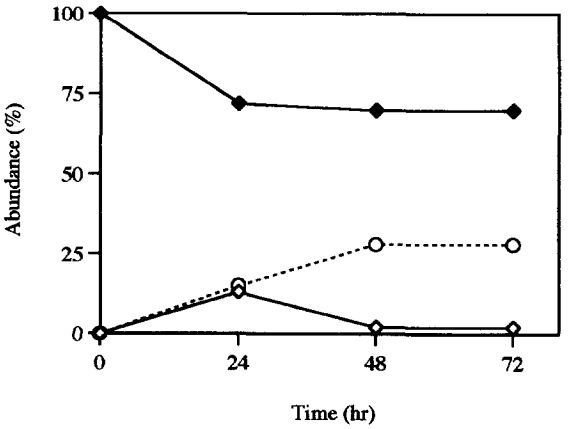

Fig. 3. Time-course of (+)-eudesmin (**1a**, ◆) and metabolites **2a** (◇) and **3a** (○).

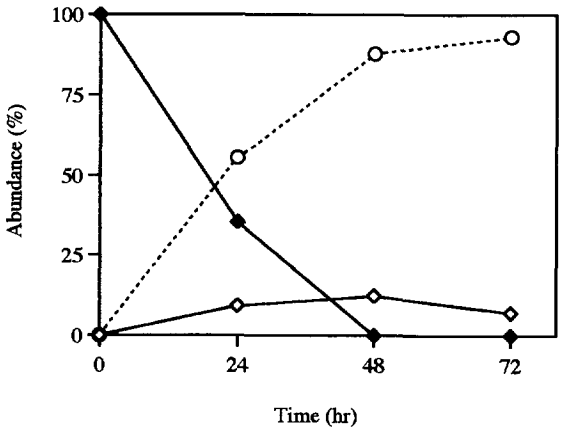

Fig. 4. Time-course of (−)-eudesmin (**1b**, ◆) and metabolites **2b** (◇) and **3b** (○).

exhausted and was converted mainly to **3b** (87.8%). The yield of **3b** amounted to 93% after 72 hr. These conversion rates were faster than that of **1a**, and of **1b** in (±)-eudesmin.

We first presumed that **3a** was obtained by enantioselective *O*-demethylation of **1**. Because most of the naturally occurring eudesmin has the (+)-configuration, there is a possibility that many organisms possess enzymes for metabolizing **1a** rather than **1b**. However, in the case of *A. niger*, it *O*-demethylated both enantiomers, accumulating **3b** enantioselectively at 25°C after 72 hr. Enantioselective accumulation of **3b** occurred by accumulation of **3b** and enantioselective metabolism of **3a**. Previously, we reported that **3a** was transformed to (+)-5′-hydroxypinoresinol by this fungus. However, its presence was not detected in the present experiments. The unidentified metabolite of **3a** must therefore be investigated in order to clarify its metabolic fate.

EXPERIMENTAL

Synthesis of (±)*-eudesmin* (**1**). Synthesized by modifying Pelter's dilactone route [12]. The key intermediate, threo-2,3-bis-(α-hydroxy-3,4-dimethoxybenzyl)butane-1,4-diol (620 mg) was stirred in 1 M HCl in dry Et_2O (20 ml) for 3 hr at room temp. The mixt. was poured into H_2O, extracted with Et_2O (100 ml × 3), then evapd and subjected to silica gel CC to give **1** (170 mg, 27.4%).

Preparation of (±)*-pinoresinol monomethyl ether* (**2**) *and* (−)*-eudesmin* (**1b**). Compound **3** [13] was stirred with CH_2N_2 for 24 hr, then **2** was sepd from **1** and unreacted **3** by silica gel CC. Compound **1b** was prepd by methylation of **3b** with CH_2N_2.

Microorganism and culture conditions. Spores of *A. niger* IFO 4414 (purchased from Institute of Fermentation of Osaka) were maintained on nutrient agar slants at 10° and inoculated into the culture medium (50 ml in a 200-ml Erlenmeyer flasks). The composition of the culture medium was as follows: sucrose 15 g, glucose 15 g, polypeptone 5 g, KCl 0.5 g, $MgSO_4 \cdot 7(H_2O)$ 0.5 g, K_2HPO_4 1g, $FeSO_4 \cdot 7(H_2O)$ 0.01 g, H_2O to 1l, it was autoclaved before inoculation. Cultivation was carried out for 2 days at 25° under static conditions. Active mycelia were transplanted to the culture medium (15 ml in a 50 ml Petri dish) and incubated for 36–42 hr (until mycelia occupied 60–80% of surface area of culture medium) under the same conditions. Compound **1** (170 mg) was dissolved in 2 ml of DMSO and added to the culture medium (corresponding to 10–20 mg of substrate per Petri dish) in the Petri dish (sterilized at 160° for 15 min). Petri dishes were incubated at 25° under static conditions, together with two control dishes which contained either mycelia with medium or substrate dissolved in DMSO with medium.

HPLC analysis. HPLC was carried out with detection at 280 nm using Daicel Chiralcel OD (250 × 4.6 mm i.d.) [14]. Chiral HPLC employed EtOH–hexane (1:1) at a flow rate of 0.8 ml min^{-1}. (+)-Pinoresinol monomethyl ether (**2a**), R_t 25.6 min, (−)-pinoresinol monomethyl ether (**2b**), R_t 30.3 min, (+)-pinoresinol (**3a**), R_t 52.2 min, (−)-pinoresinol (**3b**), R_t 23.9 min, (+)-eudesmin (**1a**), R_t 30.7 min, (−)-eudesmin (**1b**), R_t 36.5 min.

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