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Stereoselective hydrogenation on the exocyclic and conjugated double bond of sesquiterpene lactones by *Aspergillus versicolor* D-1

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Aspergillus versicolor D-1 was employed to convert dehydrocostuslactone (**1**) and 3-hydroxy-1(10),3,11(13)-guaiatriene-12,6-olide-2-one (**5**) stereoselectively. The reactions occurring were specific hydrogenation on the exocyclic α,β-double bond of sesquiterpene lactones with excellent conversion. Products were identified by the analysis of their spectra such as UV, IR, MS, ^{1}H, ^{13}C NMR, and NOESY, and the structure of one new compound was elucidated. The characteristic of the stereoselective hydrogenation was also discussed and suggested.

Keywords: *Aspergillus versicolor* D-1; dehydrocostuslactone; stereoselective hydrogenation

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1. Introduction

Biocatalysis has gained increasing interest in recent years due to its mild conditions, friendly environment, especially exquisite selectivity and high efficiency [1]. Biotransformation is an increasingly valuable tool for the production of chemicals and drugs with great potential; these modifications result in the formation of novel and useful products that are difficult to be obtained through conventional chemical procedures [2]. Moreover, the technology of biocatalysis has played an important role in enzymatic asymmetric reduction. Sesquiterpene lactones have been reported to possess noteworthy bioactivities such as anti-inflammatory [3], anti-tumor [4–6], and anti-microbial [7–9]. It was found that the transformation of sesquiterpene lactones could run regio- and stereoselectively with fungi [10]. Two sesquiterpene lactone compounds, dehydrocostuslactone (**1**) and costunolide (**8**), were isolated from the root of *Aucklandia lappa*, a kind of traditional medicinal plant in China and India, and recognized as the major components with remarkable biological activity [11,12].

Barrero *et al.* [13] reported that compound **1** could be transformed into (+)-11α,13-dihydrodehydrocostuslactone by *Cunninghamella echinulata* after a 48 h cultivation. Meanwhile, incubation of *Rhizopus oryzae* with **1** could afford two products identified as (+)-11β,13-dihydrodehydrocostuslactone and (+)-11β,13-dihydro-10,14-epoxydehydrocostuslactone, respectively. Hashimoto *et al.* [14] reported that biotransformation of compound **1** by *Aspergillus niger* afforded four kinds of metabolites for 7 and 10 days, respectively.

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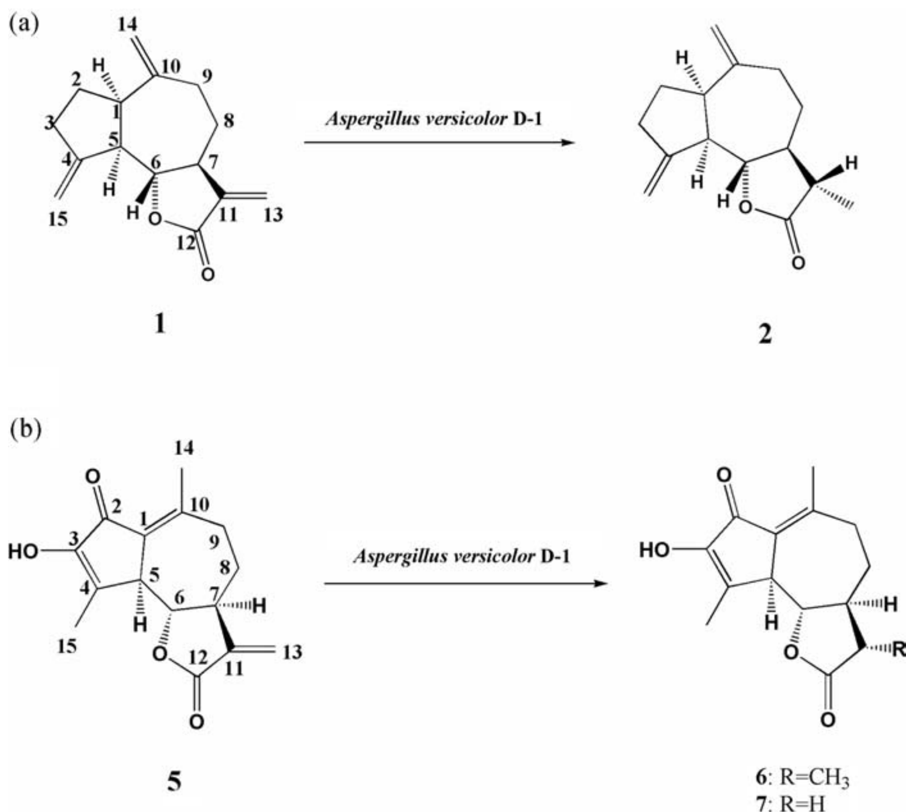

Figure 1. The reduction of compounds **1** and **5** by *A. versicolor* D-1.

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From our earlier work, it has been shown that *Aspergillus versicolor* D-1 was able to hydrogenate the γ,δ-double bond of securinine (**3**) into 14,15-dihydrosecurinine (**4**) with the conversion of over 98% (w/w) and reduce the C11(13) double bond of compound **5** to give (5*S*,6*S*,7*S*,11*S*)-3-hydroxy-1(10),3-guaiadiene-12,6-olide-2-one (**6**) with the conversion of over 98% (w/w) stereoselectively by growing cells [15]. In order to investigate the characteristic of the stereoselectivity of *A. versicolor* D-1 and obtain enantiopure compounds, further study was performed.

In this paper, *A. versicolor* D-1 was used to stereoselectively catalyze compounds **1** and **5**. The conversion of compound **1** was much better than that reported before. In a further study on compound **5**, a pair of epimers were obtained named as (5*S*,6*S*,7*S*,11*S*)-3-hydroxy-1(10),3-guaiadiene-12,6-olide-2-one (**6**) and (5*S*,6*S*,7*S*,11*R*)-3-hydroxy-1(10),3-guaiadiene-12,6-olide-2-one (**7**), respectively. Compound **7** was confirmed as a new compound (Figure 1).

2. Results and discussion

In order to elucidate the efficiency and selectivity of the hydrogenation by *A. versicolor* D-1, the comparisons of the culture results between the growing cells and resting cells were performed on compound **1**, and the selectivity was shown by calculating the diastereomeric excess (d.e.) value of the products transformed from compound **5**.

Compound **1** was converted into compound **2** at over 77% (w/w) by resting

cells for 2 h. After an additional 2 h incubation, the conversion of compound **1** reached 98.2% (w/w). The peak concentration of compound **2** could be kept in the buffer solution for more than 20 h. The product was purified and identified as 11β,13-dihydrodehydrocostuslactone using spectral measurements. The result revealed that *A. versicolor* D-1 had the ability to stereoselectively reduce the exocyclic double bond on compound **1** to give a unique product after incubation for 4 h. Compared with resting cells, the peak concentration of compound **2** (94.4% yield, w/w) will take 4 h to reach by growing cells as well; however, the concentration could just be sustained for about 2 h and then declines gradually, which was maybe because the product was metabolized by growing cells.

Compound **5** was analogous with **1**, and could be transformed into a pair of epimers by resting cells of *A. versicolor* D-1, which led to 95% yields (w/w) of **6**, together with a trace amount of **7**. Surprisingly, **7** is a new compound. The enzymatic asymmetric reduction of compound **5** could give the main product (compound **6**) with 95% d.e.

Besides, another analogue, compound **8**, which also has a similar five-membered ring lactone with the exocyclic and conjugated double bond, underwent the same procedure as described above for compounds **1** and **5**. It was interesting to find that no hydrogenation occurred on compound **8**, even if the attempt to detect the putative product was carried on until 120 h incubation with growing cells. Comparing the structure of **8** with **1** and **5**, there is a bigger 10-membered ring adjacent to five-membered lactone in it. This could suggest that the activity of hydrogenation from *A. versicolor* D-1 was not only dependent on the structure features of five-membered lactone in the substrates, but also on the attached structure next to the lactone. To understand the mechanism and target enzyme in *A. versicolor* D-1, further study is now in progress.

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3. Experimental

3.1 General experimental procedures

IR spectra in KBr disk were run on a Bruker IFS-55 spectrophotometer. ^{1}H and ^{13}C NMR spectra were generated in deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO-d_6) with Bruker spectrometers (ARX-300 and AV-600). ^{1}H–^{1}H COSY, HMQC, and HMBC spectra were generated in $CDCl_3$ on a Bruker AC 200 spectrometer. Tetramethylsilane was used as the internal standard and chemical shifts were recorded in ppm. ESI-MS was performed on a Waters micromass ZQ2000 mass spectrometer. TLC analyses were performed on silica gel 60 F_{254} plates (Merck, Darmstadt, Germany).

3.2 Microorganism and growth

A. versicolor D-1 had been isolated and identified in the laboratory. The culture medium used for growing cells ($g\,l^{-1}$) was: sucrose (15), glucose (15), polypeptone (5), K_2HPO_4 (1), $MgSO_4$ (0.5), KCl (0.5), and $FeSO_4 \cdot 7H_2O$ (0.01) in distilled water, pH 7.0.

3.3 Substrates

Compounds **1** and **5** (Figure 2) were purified from the root of *A. lappa* according to the literature [16] and compound **5** was prepared as previously described [15]. Compounds **1** and **8** were identified by their physical characters and spectral data, comparing with the literature [17]. The purity was above 98.0% by HPLC analysis.

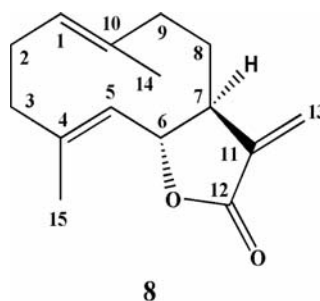

Figure 2. The structure of compound **8**.

3.4 General procedure for biotransformation of dehydrocostuslactone (1)

Biotransformation of compound **1** by both growing cells and resting cells was studied. For growing cells, a spore suspension of *A. versicolor* D-1 was first prepared and suitably diluted, and then it was inoculated into a 250 ml Erlenmeyer flask containing 50 ml liquid medium at 28°C on a rotary shaker (250 rpm) for 48 h. After that, the substrate (10 mg) dissolved in 0.5 ml of acetone was added to 50 ml liquid medium and incubated for an additional 24 h. For resting cells, *A. versicolor* D-1 was grown in an Erlenmeyer flask (250 ml) containing 50 ml medium and incubated at 250 rpm and 28°C for 48 h. The mycelia (0.2 g, dry weight) were harvested by filtration and washed with sterile distilled water, and transferred into the Erlenmeyer flask (250 ml) containing Na_2HPO_4–KH_2PO_4 buffer (0.1 M, pH 7.0, 50 ml). After adding the substrate (10 mg in 0.5 ml of acetone), the cultures were incubated for an additional 24 h.

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During the additional culture time, samples from cultures were taken periodically and analyzed by TLC and HPLC in order to determine the degree of transformation of the substrate. Culture control consisted of a fermentation blank in which the strain was grown but without the substrate. Substrate control consisted of a medium containing the substrate without the strain.

Preparative-scale transformation of compound **1** by *A. versicolor* D-1 was carried out in 1000 ml Na_2HPO_4–KH_2PO_4 buffer (0.1 M, pH 7.0, 10 × 100 ml each flask). The substrate (20 mg) in 1 ml acetone was added to each 100 ml precultured medium for 24 h. Two hundred milligrams of compound **1** in total were used. The reaction mixtures were filtered to remove the biomass. The filtrate was extracted with the same volume of ethyl acetate for three times. The organic phase was evaporated to a suitable concentration under reduced pressure and dried *in vacuo* at 60°C. The residue (356 mg) was then purified on a silica gel column (silica gel H, 2.4 × 12 cm, 10–40 μm, 15.0 g) using a mixture of petroleum ether and ethyl acetate (20:1, v/v) as the mobile phase to give pure products (35.2 mg, 17.6% yield) by means of TLC and HPLC. The products were identified on the basis of spectral data (^1H and ^{13}C NMR, HMQC, HMBC, NOESY, and ESI-MS).

3.5 General procedure for biotransformation of 3-hydroxy-1(10),3,11(13)-guaiatriene-12,6-olide-2-one (5)

Preparative-scale biotransformation of compound **5** was the same as in compound **1**. The mixture of washed mycelia (3.6 g, dry weight) and compound **5** (10 mg in 0.5 ml ethanol) were evenly distributed among 12 flasks (250 ml) containing Na_2HPO_4–KH_2PO_4 buffer (0.1 M, pH 7.0, 50 ml) and incubated at 28°C on a rotary shaker (250 rpm) for 24 h. One hundred and twenty milligrams of compound **5** in total were used. The degree of transformation of the substrate was determined by means of HPLC. The reaction mixtures were filtered and passed through a macroporous resin column. The resin was eluted by distilled water, followed by 95% (v/v) ethanol. The ethanol eluate was concentrated under reduced pressure at 60°C. The residue (253 mg) was dissolved in 5 ml methanol. Semi-preparative HPLC was carried out for preparing the transformed products.

3.6 Analytical methods

The transformation of **1** was monitored by HPLC (JASCO LC-1500 system, Japan) using a column (Dikma, Diamonsil™ C_{18}, 200 × 4.6 mm, 5 μm) with a UV detector at 30°C. Methanol–H_2O (75:25, v/v) was used as the mobile phase at 1 ml/min. The detection of **1** and its transformed product

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were carried out at 225 and 205 nm, respectively.

Compound **5** and its transformed products were monitored at 270 nm by HPLC (JASCO LC-1500 system) using a column (Dikma, Diamonsil™ C_{18}, 200 × 4.6 mm, 5 μm) with methanol–H_2O (42:58, v/v) as the mobile phase at 1.0 ml/min.

Semi-preparative HPLC was performed on a JASCO LC-1500 HPLC instrument with a Cosmosil C_{18} column (250 mm × 10 mm i.d.) and a UV-1575 detector at 30°C. The eluent was methanol–H_2O (34:66, v/v) at 2 ml/min and the detection was carried out at 270 nm. Compounds **6** (32 mg, 26.7% yield) and **7** (5 mg, 4.2% yield) were obtained, respectively.

Table 1. ^{13}C NMR spectral data for compound **1** and its reduction product (**2**) ($CDCl_3$, 150 MHz).

C	1	2
1	45.8	47.0
2	31.0	32.5
3	37.0	37.6
4	149.9	151.7
5	52.7	51.9
6	86.0	85.3
7	48.3	49.9
8	31.6	30.2
9	33.3	32.5
10	151.9	149.9
11	140.4	42.0
12	171.0	178.7
13	120.9	13.2
14	110.3	111.8
15	113.3	109.2

3.7 *Identification of the transformed products*

3.7.1 *Compound 2*

Yellow oil; $[\alpha]_D^{25}$ −13.7 ($c = 0.32$, $CHCl_3$); UV λ_{max} (MeOH): 205 nm; IR (KBr) ν_{max} (cm^{-1}): 2928, 1773; ESI-MS: *m/z* 255 $[M+Na]^+$; ^1H NMR spectral data were shown as follows: ^1H NMR (600 MHz, $CDCl_3$): δ 2.50 (1H, t, $J = 4.5$ Hz, H-2a), 2.48 (1H, t, $J = 4.7$ Hz, H-3a), 2.21 (1H, m, H-11), 2.05 (1H, m, H-3b), 1.98 (1H, m, H-7), 1.94 (1H, m, H-8a), 1.29 (1H, m, H-2b), 1.29 (1H, m, H-8b), 1.24 (3H, m, H-13). The ^{13}C NMR spectral data are shown in Table 1. The NOE experiment was used to determine the stereochemistry of the C-13 methyl group. The NOE spectrum revealed correlations between H-6 and H-11, H-7 and CH_3-13. The configuration of H-6 was β in the structure of the natural sesquiterpene lactone. All these points suggested that compound **2** was an α-configuration of C-13 methyl group and β arrangement of H-11. Therefore, the structure of compound **2** was identified as 11β,13-dihydrodehydrocostuslactone.

3.7.2 *Compound 6*

White crystal; $[\alpha]_D^{25}$ + 66.7 ($c = 0.52$, MeOH); UV λ_{max} (MeOH): 270 nm; IR (KBr) ν_{max} (cm^{-1}): 3331, 1774, 1679; ESI-MS of compound **6** showed an $[M+H]^+$ ion peak at *m/z* 263, indicating the molecular formula of $C_{15}H_{18}O_4$. ^1H

Table 2. ^1H NMR spectral data of compounds **6** and **7** ($CDCl_3$, 300 MHz).

H	6	7
5	3.26 (1H, d, $J = 11.6$ Hz)	3.29 (1H, d, $J = 10.2$ Hz)
6	3.56 (1H, t, $J = 9.9$ Hz)	3.76 (1H, t, $J = 10.2$ Hz)
7	1.95 (1H, dt)	1.85 (1H, dt)
8	1.32 (1H, dd, $J = 12.3$ Hz), 1.98 (1H, m)	1.34 (1H, m), 1.90 (1H, m)
9	2.42 (1H, m), 2.46 (1H, m)	2.35 (1H, m), 2.37 (1H, m)
11	2.24 (1H, m)	2.27 (1H, m)
13	1.28 (3H, d, $J = 6.8$ Hz)	1.13 (3H, d, $J = 7.7$ Hz)
14	2.48 (3H, s)	2.48 (3H, s)
15	2.18 (3H, s)	2.18 (3H, s)

Table 3. ^{13}C NMR spectral data of compounds **6** and **7** (DMSO-d_6, 75 MHz).

C	6	7
1	128.8	128.6
2	189.0	188.8
3	152.2	152.0
4	135.6	135.0
5	47.9	48.3
6	85.7	85.0
7	56.0	51.5
8	25.9	23.5
9	37.3	37.3
10	154.0	154.0
11	41.1	39.4
12	177.7	178.6
13	12.4	10.0
14	22.2	22.1
15	14.1	14.1

and ^{13}C NMR spectral data, see Tables 2 and 3. The NOE enhancement was observed between the proton signals of H-6 and H-11, H-7 and CH_3-13. The result confirmed that the orientation of C-13 methyl on compound **6** was α-form.

*3.7.3 Compound **7***

White crystal; $[\alpha]_D^{25} + 23.8$ ($c = 0.48$, MeOH); UV λ_{max} (MeOH): 270 nm; IR (KBr) ν_{max} (cm^{-1}): 3329, 1746, 1669; ESI-MS: *m/z* 263 $[M+H]^+$; ^{1}H and ^{13}C NMR spectral data, see Tables 2 and 3. In the NOE spectrum, the NOE enhancement was observed between the proton signals of H-6 and CH_3-13, H-7 and H-11. The result confirmed that the orientation of C-13 methyl on compound **7** was β-form.

References

[1] W.A. Loughlin, *Bioresour. Technol.* **74**, 49 (2000).

[2] K.B. Borges, W. de Souza Borges, R. Durán-Patrón, M.T. Pupo, P.S. Bonato, and I.G. Collado, *Tetrahedron: Asymmetry* **20**, 385 (2009).

[3] J.Y. Cho, K.U. Baik, J.H. Jung, and M.H. Park, *Eur. J. Pharmacol.* **398**, 399 (2000).

[4] I.H. Hall, K.H. Lee, E.C. Mar, C.O. Starnes, and T.G. Waddell, *J. Med. Chem.* **20**, 333 (1977).

[5] N. Gören, H.J. Woerdenbag, and C. Bozok-Johansson, *Planta Med.* **62**, 419 (1996).

[6] J.Y. Cho, J. Park, E.S. Yoo, K.U. Baik, J.H. Jung, J. Lee, and M.H. Park, *Planta Med.* **64**, 594 (1998).

[7] N. Gören, J. Jakupovic, and S. Topal, *Phytochemistry* **29**, 1467 (1990).

[8] N.H. Fischer, L. TianSheng, C.L. Cantrell, J.C. Castañeda-Acosta, L. Quijano, and S.G. Franzblau, *Phytochemistry* **49**, 559 (1998).

[9] C.L. Cantrell, I.S. Nuez, J. Castañeda-Acosta, M. Foroozesh, F.R. Fronczek, S.G. Franzblau, and N.H. Fischer, *J. Nat. Prod.* **61**, 1181 (1998).

[10] J. Aleu, J.R. Hanson, R.H. Galan, and I.G. Collado, *J. Nat. Prod.* **62**, 437 (1999).

[11] R.S. Dhillon, P.S. Kalsi, and W.P. Singh, *Phytochemistry* **6**, 1209 (1987).

[12] J.Y. Cho, J. Park, E.S. Yoo, K.U. Baik, J.H. Jung, J. Lee, and M.H. Park, *Planta Med.* **64**, 594 (1998).

[13] A.F. Barrero, J.E. Oltra, D.S. Raslan, and D.A. Saude, *J. Nat. Prod.* **62**, 726 (1999).

[14] T. Hashimoto, Y. Noma, and Y. Asaka, *Heterocycles* **54**, 529 (2001).

[15] H. Guan, S. You, L. Yang, X. Wang, and R. Ni, *Biotechnol. Lett.* **27**, 1189 (2005).

[16] H. Chen and X. Deng, *Chin. Tradit. Herbal Drugs* **28**, 337 (1997).

[17] B. Ferrari, P. Castilho, F. Tomi, A.I. Rodrigues, M. do-Ceu-Costa, and J. Casanova, *Phytochem. Anal.* **16**, 104 (2005).

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