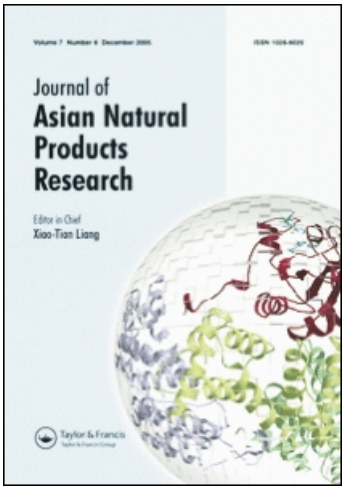

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Microbial transformation of alantolactone by *Mucor polymorphosporus*

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Two new transformed sesquiterpenes of alantolactone by *Mucor polymorphosporus* were obtained. They were characterized as 3β-hydroxy-11βH-eudesm-5-en-8β,12-olide (**2**) and 3β,11α-dihydroxy-eudesm-5-en-8β,12-olide (**3**), on the basis of spectral methods including 2D NMR. And product **3** was an unusual hydroxylation derivative of alantolactone at C-11.

Keywords: biotransformation; alantolactone; *Mucor polymorphosporus*; sesquiterpene; *Inula helenium*

1. Introduction

Inula helenium L. is an important medicinal plant and its rhizome has been used as a traditional Chinese herb [1]. Alantolactone (**1**) is one of the major active constituents in this plant with the activities of anticancer [2] and antiproliferative [3]. In our previous work [4–8], the biotransformation of a series of bioactive sesquiterpenes such as curdione, germacrone, dehydrocostuslactone, and costunolide was investigated to modify their structures and obtain some new chemical entities. In this paper, we reported the biotransformation of **1** by filamentous fungi, with the aim of improving its activities and solubility. Incubation of **1** with *Mucor polymorphosporus* for 3 days yielded only two new compounds, 3β-hydroxy-11βH-eudesm-5-en-8β,12-olide (**2**) and 3β,11α-dihydroxy-eudesm-5-en-8β,12-olide (**3**). And product **3** was an unusual hydroxylation derivative of alantolactone at C-11, and only one derivative of **1** with hydroxylation at C-11 was reported in the natural products [9].

2. Results and discussion

Incubation of alantolactone (**1**) with *M. polymorphosporus* for 3 days yielded two products (Figure 1). Their structures were identified as 3β-hydroxy-11βH-eudesm-5-en-8β,12-olide (**2**) and 3β,11α-dihydroxy-eudesm-5-en-8β,12-olide (**3**), both of which are new compounds. And product **3** was a rare hydroxylation derivative of alantolactone at C-11 in the natural products [9]. The ^{13}C-NMR spectral data of alantolactone derivative with 11-OH group were firstly reported.

According to TLC analysis, the transformed products were more polar than the substrate. Site-specific hydroxylation and hydrogenation reactions of the substrate were observed in the biotransformation process of **1**.

Compound **2** was obtained as colorless oil (MeOH). Its HR-ESI-MS provided a quasi-molecular ion $[M + H]^+$ at *m/z* 251.1642, suggesting the molecular formula of $C_{15}H_{22}O_3$. Compared with that of compound **1**, the ^{1}H-NMR spectrum of **2** showed the disappearance of two olefin protons at δ 6.21

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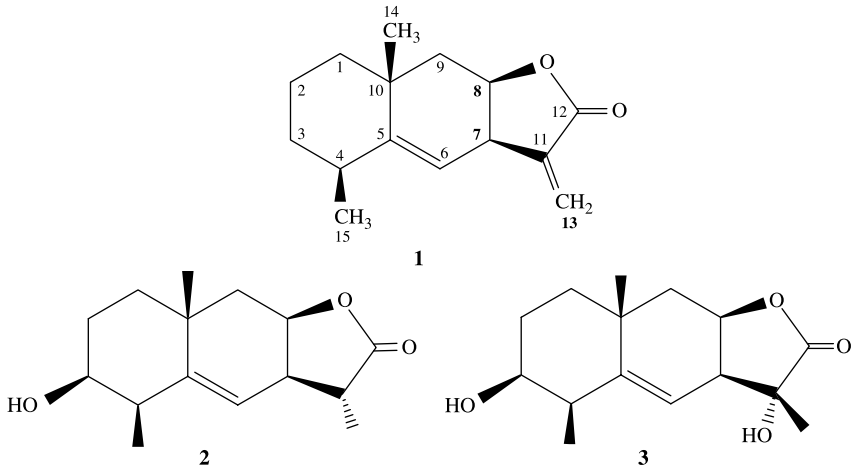

Figure 1. Chemical structures of compounds **1**–**3**.

and 5.42 in **1**, and appearance of the methyl group at δ 1.24 (d, $J = 7.0$ Hz) and a methine proton signal at δ 2.34 (m), which indicated that the carbon–carbon double bond between C_{11} and C_{13} was reduced by hydrogenation reaction. In HMBC spectrum, Me-13 at δ 1.24 had correlations with C-7 (δ 41.6), C-11 (δ 42.9), and C-12 (δ 179.4), suggesting that this methyl group was attached to C-11 of **2**, and confirmed the above deducement. NOE correlations of Me-13 (δ 1.24) with H-7 (δ 2.65) and H-8 (δ 4.91) were observed, suggesting the α-configuration of Me-13. Moreover, one oxygen-bearing methine at δ 71.2 and δ_H 3.46 was observed, and it exhibited HMBC correlations with C-1, C-5, and C-15, suggesting that the hydroxyl group at δ 4.57 was connected to C-3. Namely, compound **2** possessed a hydroxyl group at C-3. In addition, NOE enhancement between (i) 3-OH (δ 4.57) and H-15 (δ 0.99); (ii) H-3 (δ 3.46) and H-4 (δ 2.46) were observed, all of which indicated that 3-OH should be in β-configuration. On the basis of the above analysis, compound **2** was identified as 3β-hydroxy-11βH-eudesm-5-en-8β,12-olide. All ^{1}H- and ^{13}C-NMR spectral data were unambiguously assigned by 2D NMR spectra (Table 1).

Compound **3** was obtained as colorless oil (MeOH). Its HR-ESI-MS provided a quasi-molecular ion $[M + H]^+$ at *m/z* 267.1591, suggesting the molecular formula of $C_{15}H_{22}O_4$. The ^{1}H-NMR spectrum of **3** closely resembled that of **2**. And in the ^{13}C-NMR spectrum of **3**, the C-13 shifted downfield to δ 20.1, and a new oxygen-bearing carbon (δ 76.0) was observed, when compared to **2**. This evidence indicated that **3** was a hydroxylation product of **2**. In HMBC spectrum, Me-13 (δ 1.24) correlated with C-12 (δ 177.1), C-7 (δ 46.3), and C-11 (δ 76.0), suggesting that the hydroxyl group were introduced in C-11. NOE correlations of 11-OH with H-7 and H-8 were observed, indicating that 11-OH should be in α-configuration. On the basis of the above analysis, compound **3** was identified as 3β,11α-dihydroxy-eudesm-5-en-8β,12-olide. To our knowledge, the derivative of alantolactone with 11-OH was rare, and only one hydroxylation product of **1** at C-11 was reported in the natural products [9]. All ^{1}H- and ^{13}C-NMR spectral data were unambiguously assigned by 2D NMR spectra (Table 2).

3. Experimental

3.1 General experimental procedures

Optical rotations were obtained with a Perkin-Elmer 243B polarimeter. UV spectra were

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data of **2** in DMSO-d_6 (δ in ppm, J in Hz).

No.	^{1}H multiplicity (J, Hz, DMSO)	^{13}C DMSO	HMBC (H → C)	NOESY
1a	1.51 m	39.2	C-2, C-3, C-10	H-2a, H-14
1b	1.15 m			H-2b
2a	1.66 m	25.4	C-1, C-3, C-4	H-1a, H-14
2b	1.42 m			H-1b, H-3
3	3.46 m, 4.57 m (OH)	71.2	C-1, C-2, C-4, C-5, C-15 C-2, C-3, C-4	H-2b, H-4, H-3, H-15
4	2.46 m	44.8	C-2, C-3, C-5, C-10, C-15	H-3, H-15
5	–	147.1	–	–
6	5.24 d, $J = 3.0$	121.7	C-4, C-7, C-8	H-7
7	2.65 m	41.6	C-6, C-8, C-11, C-12	H-6, H-8, H-13
8	4.91 m	76.0	C-6, C-7, C-9	H-7, H-13
9a	1.98 m	42.1	C-5, C-7, C-8, C-14	H-9b, H-14
9b	1.45 m			H-9a
10	–	32.0	–	–
11	2.34 m	42.9	C-6, C-7, C-8, C-12, C-13	H-13
12	–	179.4	–	–
13	1.24 d, $J = 7.0$	15.8	C-7, C-11, C-12	H-7, H-8, H-11
14	1.11 s	28.8	C-1, C-9, C-10	H-9a
15	0.99 d, $J = 7.0$	15.9	C-3, C-4, C-5	OH-3, H-4

measured on a YV-1091 UV–vis spectrophotometer. IR spectra were recorded on an Avatar 360 FT-TR spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR) in DMSO-d_6 with TMS as internal standard. HR-MS was obtained on a Bruker APEXII Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer. Silica gel (200–300 mesh) was purchased from Qingdao Marine Chemical Group, Qingdao, China. Alantolactone (**1**) was isolated from *I. helenium* L. by the author. The purity was above 98% determined by

Table 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data of **3** in DMSO-d_6 (δ in ppm, J in Hz).

No.	^{1}H multiplicity (J, Hz, DMSO)	^{13}C DMSO	HMBC (H → C)	NOESY
1a	1.51 m	39.5	C-3, C-10	H-1b, H-14
1b	1.13 m			H-1a
2a	1.64 m	25.3	C-1, C-3, C-4	H-2b, H-14, H-15
2b	1.44 m			H-2a
3	3.49 m, 4.58 m (OH)	71.0	C-1, C-2, C-4	H-4, H-3, H-15
4	2.49 m	45.0	C-2, C-3, C-5, C-10, C-15	H-3
5	–	149.1	–	–
6	5.18 (d, $J = 3.0$)	117.5	C-7, C-8, C-10	H-13
7	2.73 m	46.3	C-6, C-8, C-11, C-12	H-8, H-11
8	4.95 m	75.6	–	H-7
9a	2.00 m	42.0	C-5, C-7, C-8, C-10, C-14	H-9b, H-14
9b	1.48 m			H-9a
10	–	31.7	–	–
11	6.03 s (OH)	76.0	C-7, C-11, C-12	H-7, H-8, H-13
12	–	177.1	–	–
13	1.24 s	20.1	C-7, C-11, C-12	H-6, 11-OH
14	1.05 s	28.6	C-1, C-5, C-9, C-10	H-2a, H-9a
15	0.95 (d, $J = 7.0$)	16.2	C-3, C-4, C-5	H-2a, 3-OH, H-4

HPLC. Its structure was characterized by ^{1}H NMR, ^{13}C NMR, and EI-MS.

3.2 Microorganisms

Mucor spinosus AS 3.3450, *M. spinosus* AS 3.2450, *M. spinosus* AS 3.3447, *Mucor subtilissimus* AS 3.2454, *M. subtilissimus* AS 3.2456, *M. polymorphosporus* AS 3.3443, *Cunninghamella blakesleana lender* AS 3.970, *Cunninghamella elegans* AS 3.1207, *C. elegans* AS 3.2028, *Alternaria alternata* AS 3.577, *A. alternata* AS 3.4578, *Alternaria longipes* AS 3.2875, *Penicillium melinii* AS 3.4474, *Penicillium janthinellum* AS 3.510, *Syncephalastrum racemosum* AS 3.264, *Trichoderma viride* AS 3.2942, *Rhizopus stolonifer* AS 3.3463, *R. stolonifer* AS 3.2050, *Rhizopus arrhizus* AS 3.2897, and *Curvularia lunata* AS 3.4381 were purchased from China General Microbiological Culture Collection Center in Beijing, China.

3.3 Culture medium

All cultures of filamentous fungi were performed in potato medium that was made of the following composition (l): 200 g potato and 20 g glucose [10].

3.4 Screening test

Twenty strains of filamentous fungi were used to screen for their capabilities to transform **1**. The flasks containing 100 ml of the liquid culture were placed on the rotary shakers, operating at 180 rpm at 28°C. The substrates were dissolved in acetone to reach a concentration of 10 mg/ml. After 36 h of pre-culture, 0.2 ml of the solution was added into each flask and these flasks were maintained under the fermentation condition for 3 days. Culture controls consisted of fermentation blanks in which microorganisms were grown without substrate but with the same amount of acetone. Substrate controls contained the sterile medium with the same amount of substrate and were incubated under the above conditions.

3.5 Preparative biotransformation *by* M. polymorphosporus

Preparative-scale biotransformation of alantolactone (**1**) by *M. polymorphosporus* was carried out in a 1000 ml Erlenmeyer flask. The substrate (15 mg) in 1 ml acetone was added to 350 ml medium for 48 h. In total, 180 mg of substrate was used. The incubation was continued for three additional days.

The culture liquid was filtered and then partitioned with the same volume of ethyl acetate for five times. The organic phase was collected and concentrated to dryness *in vacuo*. The residues were applied to silica gel column and eluted with petroleum ether–acetone (in a gradient manner from 100:1 (v/v) to 1:2, at a flow rate of 2.0 ml/min) to obtain fractions I–V. Fraction IV was applied to preparative HPLC (CH_3CN–H_2O 15:85 (v/v)) to furnish **2** (50 mg). Fraction V was applied to preparative HPLC (CH_3CN–H_2O 25:75 (v/v)) to obtain **3** (4 mg).

3.6 Structural identification

3β-Hydroxy-11βH-eudesm-5-en-8β,12-olide (**2**): colorless oil (Et_2O); $[\alpha]_D^{22}$ +30.6 (*c.* 0.2, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3283, 2878, 1765, 1378, 1033. UV λ_{max} MeOH: 201.0 nm; ^{1}H-NMR and ^{13}C-NMR spectral data see Table 1. HR-ESI-MS (*m/z*): 251.1642 $[M + H]^+$ (calcd for $C_{15}H_{23}O_3$, 251.1646).

3β,11α-Dihydroxy-eudesm-5-en-8β,12-olide (**3**): colorless oil (Et_2O); $[\alpha]_D^{22}$ +9.1 (*c.* 0.2, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3277, 2968, 1749, 1215, 1039. UV λ_{max} MeOH: 205.0 nm; ^{1}H-NMR and ^{13}C-NMR spectral data see Table 2. HR-ESI-MS (*m/z*): 267.1591 $[M + H]^+$ (calcd for $C_{15}H_{23}O_4$, 267.1598).

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