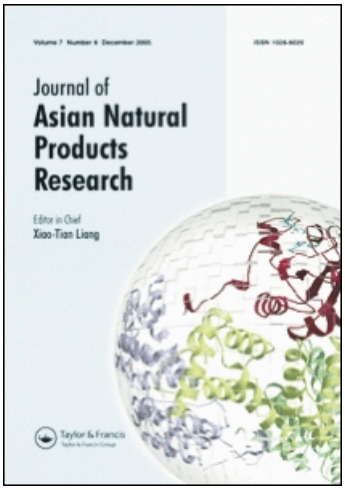


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# Microbial transformation of dehydrocostuslactone by *Mucor polymorphosporus*

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Biotransformation of dehydrocostuslactone **(1)** by *Mucor polymorphosporus* yielded four compounds, and their structures were identified as 11α,13-dihydrodehydrocostuslactone **(2)**, 3α-hydroxy-11α,13-dihydrodehydrocostuslactone **(3)**, 3β-hydroxy-4β,15,11α,13-tetrahydrodehydrocostuslactone **(4)** and 2β-hydroxyl-11α,13-dihydrodehydrocostuslactone **(5)**, respectively, on the basis of their spectral data. Among them, compound 5 is a new compound.

*Keywords*: Biotransformation; Dehydrocostuslactone; *Mucor polymorphosporus*; *Aucklandia lappa*

## 1. Introduction

*Aucklandia lappa* is a commonly used medicinal plant and its rhizome has been used as a traditional Chinese medicine possessing anti-cancer, anti-oxidation and anti-fungus activities [1,2]. Dehydrocostuslactone **(1)**, with a guaiane skeleton, is one of the major active constituents in this medicinal herb. Recently, Lee *et al.* reported that dehydrocostuslactone inhibited the expression of inducible nitric oxide (NO) syntheses and tumour necrosis factor-α in lipopolysaccharide-activated macrophages [3]. Biotransformation is a useful tool to modify structures of biologically active compounds. In recent years, applying microbial transformation for studying the metabolism of natural products has been approached [4,5]. We have also fulfilled a series of biotransformation studies to optimise the structures of bioactive natural products and to pursue the mammalian metabolism by a microbial approach [6–16]. In this paper, we report the biotransformation of 1 by filamentous fungi, with the aim of improving its solubility and activities.

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## 2. Results and discussion

Incubation of dehydrocostuslactone with *Mucor polymorphosporus* for 4 days yielded four products (figure 1), their structures being identified as 11α,13-dihydrodehydrocostuslactone **(2)**, 3α-hydroxy-11α,13-dihydrodehydrocostuslactone **(3)**, 3β-hydroxy-4β,15,11α,13-tetrahydrodehydrocostuslactone **(4)** and 2β-hydroxy-11α,13-dihydro-dehydrocostuslactone **(5)**, respectively, on the basis of their spectral data. Among them, **5** is a new compound.

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

EI-MS of compound **5** showed a molecular ion peak at *m/z* 248 $[M]^+$, together with its $^1H$ NMR and $^{13}C$ NMR data, indicating the molecular formula of $C_{15}H_{20}O_3$. The methylene signals of C-13 were also replaced by a methyl doublet. The NOE enhancement between H-13 and H-6 suggested that the relative configuration of 13-Me should be in β-configuration. In comparison with that of compound **2**, the $^{13}C$ NMR spectrum of **5** exhibited an additional oxygen-bearing methine signal at $\delta$ 73.8, and the HSQC spectrum exhibited the disappearance of a methylene, suggesting the introduction of a hydroxyl group at methylene in the molecule. In the HMBC spectrum, H-2 ($\delta$ 4.09) correlated with C-3 ($\delta$ 42.4), C-5 ($\delta$ 49.8), C-1 ($\delta$ 55.4), C-10 ($\delta$ 147.8) and C-4 ($\delta$ 151.7); meanwhile $H_2$-15 ($\delta$ 5.06 and 4.93) correlated with carbon signals at C-3 ($\delta$ 42.4) and C-5 ($\delta$ 49.8), suggesting the hydroxyl group should be at C-2 position (see figure 2). The NOE

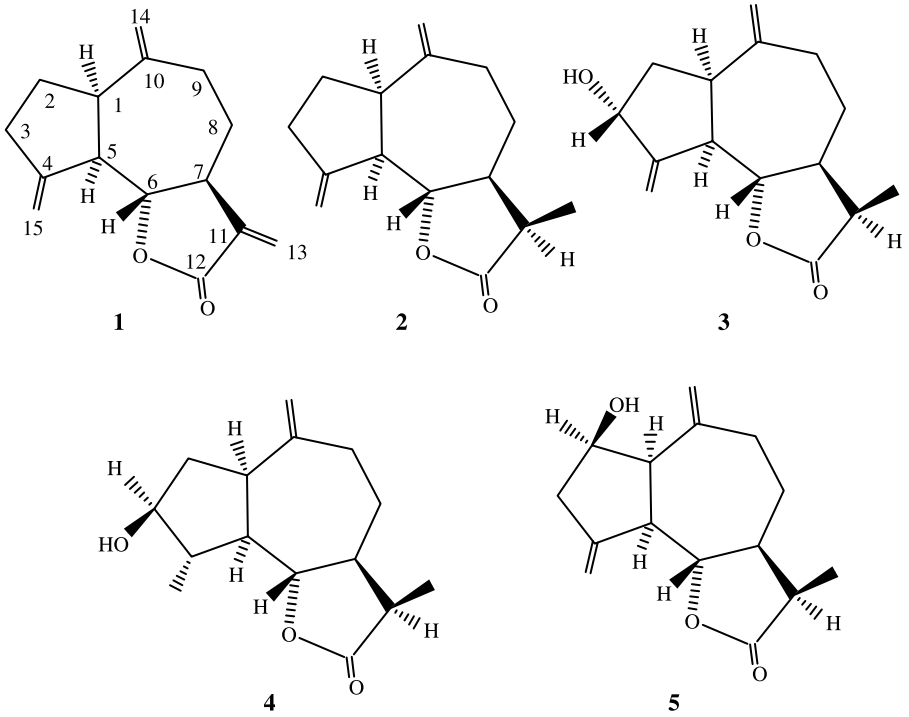
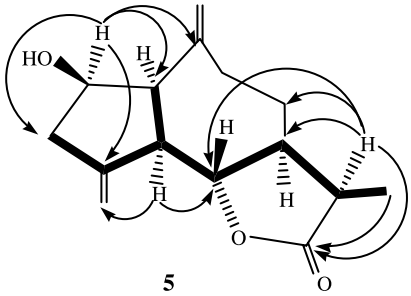



Figure 1. Chemical structures of compounds **1–5**.




**5**

Figure 2. Important HMBC correlations of compound **5**.



enhancements between H-2 ($\delta$ 4.09), H-1 ($\delta$ 2.67) and H-5 ($\delta$ 2.94) were observed in the NOE experiment. On the basis of above analysis, compound **5** was identified as 2β-hydroxy-11α,13-dihydrodehydrocostuslactone.

All the $^{1}$H NMR and $^{13}$C NMR spectral data of known compounds **2–4** were also unambiguously assigned by 2D NMR spectra, and compared with spectral data of literature values [17–19].

## 3. Experimental

### *3.1 General experimental procedures*

Melting points were determined on an XT4A apparatus and were uncorrected. Optical rotations were measured with a Perkin-Elmer 243B polarimeter. UV spectra were detected on a YV-1091 UV-Vis spectrophotometer. IR spectra were obtained 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. EI-MS data were obtained by a MS-QP5050A mass spectrometer. An Agilent 1100 series HPLC equipped with Diode array detector at 210 nm was used. Silica gel (200-300 mesh) was purchased from Qingdao Marine Chemical Group, China. Dehydrocostuslactone **(1)** was isolated from *Aucklandia lappa* by the authors. The purity was above 98% determined by HPLC.

### *3.2 Micro-organisms*

*Mucor spinosus* AS 3.3450, *Mucor spinosus* AS 3.2450, *Mucor spinosus* AS 3.3447, *Mucor subtilissimus* AS 3.2454, *Mucor subtilissimus* AS 3.2456, *Mucor polymorphosporus* AS 3.3443, *Cunninghamella blakeseana lender* AS 3.970, *Cunninghamella elegans* AS 3.1207, *Cunninghamella elegans* AS 3.2028, *Alternaria alternata* AS 3.577, *Alternaria 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, *Rhizopus stolonifer* AS 3.2050, *Rhizopus arrhizus* AS 3.2897 and *Curvularia lunata* AS 3.4381 were purchased from the China General Microbiological Culture Collection Centre in Beijing, P.R. China.

Table 1. $^{1}$H NMR data of compounds **2**, **3**, **4** and **5** (in DMSO-$d_6$, 500 MHz).

| *$^{1}$H* | ***2*** | ***3*** | ***4*** | ***5*** |
|---|---|---|---|---|
| 1 | 2.90 (1H, m) | 2.96 (1H, m) | 2.65 (1H, m) | 2.67 (1H, m) |
| 2α | 1.82 (1H, m) | 1.67 (1H, m) | 1.54 (1H, m) | 4.09 (1H, m) |
| 2β | 1.90 (1H, m) | 2.00 (1H, m) | 1.92 (1H, m) | – |
| 3α | 2.53 (1H, m) | – | 3.46 (1H, m) | 2.32 (1H, m) |
| 3β | 2.53 (1H, m) | 4.46 (1H, br.s) | – | 2.78 (1H, m) |
| 4 | – | – | 1.64 (1H, m) | – |
| 5 | 2.79 (1H, m) | 2.94 (1H, m) | 1.83 (1H, m) | 2.94 (1H, m) |
| 6 | 4.01 (1H, t, $J = 9.5$ Hz) | 3.93 (1H, t, $J = 9.5$ Hz) | 3.93 (1H, t, $J = 9.5$ Hz) | 3.92 (1H, t, $J = 9.5$ Hz) |
| 7 | 2.42 (1H, m) | 2.48 (1H, m) | 2.24 (1H, m) | 2.00 (1H, m) |
| 8α | 1.39 (1H, m) | 1.23 (1H, m) | 1.21 (1H, m) | 1.27 (1H, m) |
| 8β | 1.76 (1H, m) | 1.81 (1H, m) | 1.81 (1H, m) | 2.05 (1H, m) |
| 9α | 1.99 (1H, m) | 1.94 (1H, m) | 1.80 (1H, m) | 2.02 (1H, m) |
| 9β | 2.42 (1H, m) | 2.43 (1H, m) | 2.48 (1H, m) | 2.41 (1H, m) |
| 11 | 2.62 (1H, dq, $J = 8.0$, 8.0 Hz) | 2.61 (1H, dq, $J = 8.0$, 8.0 Hz) | 2.61 (1H, dq, $J = 8.0$, 8.0 Hz) | 2.62 (1H, dq, $J = 8.0$, 8.0 Hz) |
| 13 | 1.05 (1H, d, $J = 7.5$ Hz) | 1.03 (3H, d, $J = 7.5$ Hz) | 1.01 (3H, d, $J = 7.5$ Hz) | 1.09 (3H, d, $J = 7.5$ Hz) |
| 14a | 4.84 (1H, br.s) | 4.85 (1H, br.s) | 4.86 (1H, br.s) | 4.53 (1H, br.s) |
| 14b | 4.71 (1H, br.s) | 4.67 (1H, br.s) | 4.83 (1H, br.s) | 4.84 (1H, br.s) |
| 15a | 5.04 (1H, br.s) | 5.15 (1H, br.s) | 1.07 (3H, d, $J = 7.5$ Hz) | 5.06 (1H, br.s) |
| 15b | 4.97 (1H, br.s) | 5.12 (1H, br.s) | – | 4.93 (1H, br.s) |
| OH | – | 4.97 (br.s) | 4.77 (br.s) | 4.53 (br.s) |

### *3.3 Culture medium*

All culture and biotransformation experiments using filamentous fungi were performed in potato medium, which was made by the following composition (l): 200 g potato and 20 g glucose.

### *3.4 Screening test*

Twenty strains of filamentous fungi were screened for their capabilities to transform **1**, which were carried out in 250-ml Erlenmeyer flasks containing 100 ml of the liquid culture. The flasks were placed on rotary shakers, operating at 180 rpm at 26–28°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 4 days. Culture controls consisted of fermentation blanks in which micro-organisms were grown without substrate but with the same amount of acetone. Substrate controls contained the sterile medium with the same amount of substrate and incubated under the above conditions.



### *3.5 Preparative biotransformation by M. polymorphosporus*

Preparative scale biotransformation of dehydrocostuslactone by *M. polymorphosporus* was carried out in a 1000-ml Erlenmeyer flask. The substrate (10 mg) in 1 ml acetone was added to 350 ml pre-cultured medium for 36 h. In total, 400 mg of substrate was used. The incubation was continued for four additional days. Other procedures were the same as for screening scale biotransformation.

The culture was filtered and the filtrate extracted with same volume of EtOAc for five times. The organic phase was collected and concentrated to dryness in vacuo. The residues were applied to a silica gel column and eluted with petroleum ether/ethyl acetate (in a gradient manner from 100:3 [v/v] to 1:1, at a flow rate of 1.5 ml/min) to obtain compounds **1** (61 mg), **2** (50 mg), **3** (13 mg), **4** (15 mg) and **5** (4 mg), respectively. All of the products were identified on the basis of their spectral data.

Table 2. $^{13}$C NMR chemical shifts of the compounds **2**, **3**, **4** and **5** (in DMSO-$d_6$, 125 MHz).

| *Carbons* | ***2*** | ***3*** | ***4*** | ***5*** |
|---|---|---|---|---|
| 1 | 46.3 | 42.6 | 41.4 | 55.4 |
| 2 | 28.0 | 39.5 | 38.2 | 73.8 |
| 3 | 32.0 | 72.6 | 76.6 | 42.4 |
| 4 | 150.3 | 155.6 | 46.4 | 151.7 |
| 5 | 51.9 | 49.6 | 50.1 | 49.8 |
| 6 | 84.5 | 84.9 | 85.7 | 85.2 |
| 7 | 43.3 | 43.6 | 46.4 | 47.9 |
| 8 | 29.4 | 28.2 | 28.6 | 31.2 |
| 9 | 36.9 | 37.5 | 36.3 | 38.1 |
| 10 | 152.4 | 150.1 | 150.1 | 147.8 |
| 11 | 38.5 | 38.5 | 38.6 | 40.2 |
| 12 | 179.4 | 179.5 | 179.3 | 178.3 |
| 13 | 11.0 | 11.0 | 10.9 | 12.8 |
| 14 | 111.2 | 110.1 | 111.5 | 111.6 |
| 15 | 108.1 | 111.4 | 18.2 | 108.3 |

### *3.6 Structural identification*

**3.6.1 Compound 2**. Colourless oil ($Et_2O$). $[\alpha]_D^{22}$ + 90 (*c* 0.6, $CHCl_3$). UV $\lambda_{max}$ (MeOH): 210. $^1H$ NMR and $^{13}C$ NMR data: see tables 1 and 2.

**3.6.2 Compound 3**. Colourless oil ($Et_2O$). $[\alpha]_D^{22}$ + 28.2 (*c* 2.0, $CHCl_3$). UV $\lambda_{max}$ (MeOH): 210. $^1H$ NMR and $^{13}C$ NMR data: see tables 1 and 2.

**3.6.3 Compound 4**. Colourless oil ($Et_2O$). $[\alpha]_D^{22}$ + 35.2 (*c* 1.8, $CHCl_3$). UV $\lambda_{max}$ (MeOH): 210. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3400, 1758, 1620, 870, 810. $^1H$ NMR and $^{13}C$ NMR data: see tables 1 and 2. EI-MS (*m/z*): 250 $[M]^+$, 105, 91, 80. HRFAB-MS (*m/z*): 249.1503 $[M - H]^+$ (calcd for $C_{15}H_{21}O_3$, 249.1489).

**3.6.4 Compound 5**. Colourless oil ($Et_2O$). $[\alpha]_D^{22}$ + 21.2 (*c* 2.0, $CHCl_3$). UV $\lambda_{max}$ (MeOH): 210. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3460, 1720, 1610, 890, 810. $^1H$ NMR and $^{13}C$ NMR data: see tables 1 and 2. EI-MS (*m/z*): 248 $[M]^+$, 174, 105, 91, 55. HRFAB-MS (*m/z*): 247.1341 $[M - H]^+$ (calcd for $C_{15}H_{19}O_3$, 247.1334).



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