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Stereo- and regiospecific biotransformation of curcumenol by four fungal strains

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ABSTRACT

Biotransformations of curcumenol (**1**) were performed by four fungal strains, *Mucor spinosus* AS 3.2450, *Penicillium urticae* IFFI 04015, *Cunninghamella echinulata* AS 3.3400, *Aspergillus carbonarius* IFFI 02087. Five metabolites were prepared in the biotransformation process of **1**, and their structures were elucidated as 15-hydroxycurcumenol (**2**), 1α-hydroxycurcumenol (**3**), 14-hydroxycurcumenol (**4**), 3β-hydroxycurcumenol (**5**) and 12-hydroxycurcumenol (**6**) by spectroscopic data analysis. Among them, metabolites **2–5** are novel. All of these four fungal strains showed the ability of highly stereo- and regiospecific hydroxylation for the substrate (**1**), which could be used as tools for preparing the hydroxylated derivatives and *in vivo* metabolites of curcumenol. In addition, the inhibitory effects of substrate and obtained products on nitric oxide production in lipopolysaccaride-activated macrophages were evaluated. The substrate (**1**) and metabolites **2**, **5**, and **6** showed significant inhibitory effects.

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1. Introduction

Rhizoma Curcumae is an important crude drug frequently listed in prescriptions of traditional Chinese medicine for the treatment of Oketsu syndromes [1] which are caused by the obstruction of blood circulation, such as arthralgia, psychataxia, and dysmenorrhea. Three species of Rhizoma Curcumae (*Curcuma phaeocaulis* Valeton, *C. kwangsiensis* S.G. Lee & C.F. Liang, and *C. wenyujin* Y.H. Chen & C. Ling) are officially recorded as traditional Chinese medicine in the Chinese Pharmacopoeia [2]. Sesquiterpenoids and diarylheptanoids [3–8] are considered as the main bioactive constituents of Rhizoma Curcumae, possessing anti-inflammatory [8–10], anti-tumor [11–13], antioxidant [14,15], vasorelaxant [16], hepatoprotective [17] and neuroprotective [18] activities.

Curcumenol is one of the major guaiane-type sesquiterpenoid constituents of Rhizoma Curcumae, having an especially high content in the species of *C. phaeocaulis* Valeton [8]. Our previous studies indicated that curcumenol displayed strong inhibitory effects on nitric oxide production [8], which could be considered as a potential anti-inflammatory agent. However, the poor water-solubility would limit its bioavailability, absorption and clinical application [19,20]. Up to now, no any investigation on the structural derivatization of curcumenol has been reported owing to its limited reaction points for chemical modification.

Microbial transformation provides an important tool for structure modification of natural products. And many new chemical derivatives with potent bioactivities and improved physicochemical characteristics were found in the process of biotransformation [21,22]. Microbial transformation can also be used as an *in vitro* model to prepare the *in vivo* metabolites [23,24]. This method exhibited many advantages, such as its high stereo- and regioselectivity, ease of handling, low cost, and environmental-friendly nature [25–28].

In the present work, the biocatalysis ability of 30 fungal strains to convert curcumenol (**1**) was screened in an attempt to obtain new and bioactive derivatives of sesquiterpenoid and improve its physicochemical properties. Four fungal strains, *Mucor spinosus* AS 3.2450, *Penicillium urticae* IFFI 04015, *Cunninghamella*

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echinulata AS 3.3400 and *Aspergillus carbonarius* IFFI 02087 were chosen because they displayed the characteristics of high stereo- and regioselectivity for the biotransformations of curcumenol. Five metabolites, four of which have not previously been reported, were isolated and identified in the biotransformation processes of **1**. In addition, the inhibitory effects of the substrate and obtained products on nitric oxide production in lipopolysaccaride-activated macrophages were also evaluated.

2. Experimental

2.1. General experimental procedures

The NMR spectra were performed on Bruker ARX-600 spectrometer, using TMS as internal standard. Chemical shifts were expressed in δ (ppm) and coupling constants (J) were reported in Hertz (Hz). Optical rotation values were measured on a Perkin-Elmer 241 MC polarimeter. UV spectra were measured with a Shimadzu UV-1700 spectrophotometer. IR spectra were recorded with a Bruker IFS 55 spectrometer. HRESIMS spectra were obtained on an Agilent 6210 TOF mass spectrometer, in m/z. Melting points were determined with an X-5 hot stage microscope melting point apparatus (uncorrected). Silica gel GF254 prepared for TLC and silica gel (200–300 mesh) for column chromatography (CC) were obtained from Qingdao Marine Chemical Factory (Qingdao, People's Republic of China). Sephadex LH-20 was a product of Pharmacia. All the analytic reagents were analytical grade and purchased from Tianjin DaMao Chemical Company (Tianjin, China). Spots were detected on TLC plates under UV light or by heating after spraying with anisaldehyde-H_2SO_4 reagent.

2.2. Substrates

Curcumenol was isolated from the rhizomes of *C. phaeocaulis* by the author (Jiang-Hao Ma), and was characterized by comparison of the NMR data with those reported in Refs. [8,29]. Its purity was determined to be 98% by HPLC analysis.

2.3. Microorganism

Absidia spinosa AS 3.3391, *A. coerulea* AS 3.3382, *Alternaria alternata* AS 3.4578, *A. alternata* AS 3.577, *A. longipes* AS 3.2875, *Aspergillus niger* AS 3.739, *A. niger* AS 3.795, *A. avenaceus* AS 3.4454, *A. carbonarius* IFFI 02087, *A. candidus* IFFI *02360*, *A. flavus* AS 3.3554, *Cunninghamella elegans* AS 3.1207, *C. echinulata* AS 3.3400, *C. blakesleana* lender AS 3.970, *Fusarium avenaceum* AS 3.4594, *Mucor polymorphosporus* AS 3.3443, *M. subtilissimus* AS 3.2454, *M. spinosus* AS 3.3450, *M. spinosus* AS 3.2450, *Paecilomyces varioti* IFFI 04024, *Penicillium adametzii* AS 3.4470, *P. janthinellum* AS 3.510, *P. notatum* IFFI 04013 *P. urticae* IFFI 04015, *P. melinii* AS 3.4474, *Rhizopus chinensis* IFFI 03043, *R. stolonifer* AS 3.2050, *Sporotrichum* sp. AS 3.2882, *Syncephalastrum racemosum* AS 3.264, and *Trichoderma viride* AS 3.2942 were purchased from China General Microbiological Culture Collection Center in Beijing, China. They were screened for their abilities to transform curcumenol (**1**) in the preliminary test.

2.4. Medium

All culture and biotransformation experiments were performed in potato medium as following procedure: 200 g of minced husked potato was boiled in water for 1 h, then the extract was filtered and the filtrate was added with water to 1 L after addition of 20 g of glucose. The broth was autoclaved in individual Erlenmeyer flask at 121 °C and 15 psi for 20 min and cooled before incubation.

2.5. Biotransformation procedures

The four fungal strains (*Mucor spinosus* AS 3.2450, *Penicillium urticae* IFFI 04015, *Cunninghamella echinulata* AS 3.3400 and *Aspergillus carbonarius* IFFI 02087) had been sub-cultured for three times on potato dextrose agar slants before use to obtain maximal enzyme activities. Preliminary screening biotransformations of curcumenol (**1**) by microorganisms were carried out in 250 mL Erlenmeyer flasks containing 100 mL of potato medium. The flasks were placed on rotary shakers, operating at 180 rpm and 28 °C. After 1 day of culture, the acetone (0.2 mL) containing curcumenol (2 mg) was added into each biotransformation flask, and the biotransformations were continued under the same conditions for an additional 5 days. Both substrate and organism controls were incubated under the same conditions in order to demonstrate that substrate was stable in the control culture. Similarly, preparative scale biotransformations were carried out in 500 mL Erlenmeyer flasks containing 200 mL of potato medium, and the microorganisms were pre-cultured under culture conditions for 2 days, respectively. The acetone (0.5 mL) containing curcumenol (5 mg) was added into each biotransformation flask, and the biotransformations were continued under the same conditions for an additional 7 days. A total of 200 mg of **1** was transformed by the four strains, respectively. When the biotransformation finished, the broths of substrate were filtered and the filtrates were extracted with the equal volume of ethyl acetate for three times, respectively. The organic phase was collected and concentrated to dry under reduced pressure at 40 °C for further isolation.

2.6. Isolation and purification of metabolites

The crude transformation residues of curcumenol (**1**) by *M. spinosus* AS 3.2450 were subjected to column chromatography (CC) over silica gel and eluted with the mixtures of petroleum ether–acetone (100:1, 50:1, 20:1, 15:1, 6:1, 2:1), which yielded ten fractions (1–10). Fraction 6 was purified by preparative TLC (CH_2Cl_2/EtOAc/petroleum ether, 1.2:1:0.5) to afford **2** (11.2 mg, 5.6%) and **6** (16.6 mg, 8.3%). Fraction 9 was submitted to a silica gel column eluted with petroleum ether–ethyl acetate (20:1, 15:1, 10:1, 5:1) to give **3** (156.6 mg, 78.3%).

The crude transformation residues of curcumenol (**1**) by *P. urticae* IFFI 04015 were submitted to silica gel CC and eluted with the mixtures of petroleum ether–acetone (50:1, 30:1, 15:1, 10:1, 6:1, 3:1, 1:1), which yielded twelve fractions (1–12). Fraction 5 was purified by preparative TLC (CH_2Cl_2/EtOAc/petroleum ether, 1.2:1:0.1) to obtain **2** (137.6 mg, 68.8%) and **6** (36.4 mg, 18.2%). Fraction 6 was purified by preparative TLC (CH_2Cl_2/EtOAc/petroleum ether, 1.2:1:0.5) to afford **4** (7.8 mg, 3.9%).

The crude transformation residues of curcumenol (**1**) by *C. echinulata* AS 3.3400 were subjected to silica gel CC and eluted with the mixtures of petroleum ether–ethyl acetate (30:1, 25:1, 20:1, 15:1, 10:1, 5:1, 3:1, 1:1), which yielded fourteen fractions (1–14). Fraction 6 was chromatographed on a Sephadex LH-20 column eluted with MeOH to yield **5** (47.6 mg, 23.8%). Fraction 7 was purified by preparative TLC (CH_2Cl_2/EtOAc/petroleum ether, 1.2:1:0.5) to afford **6** (108.0 mg, 54.0%) and **2** (20.2 mg, 10.1%).

The crude transformation residues of curcumenol (**1**) by *A. carbonarius* IFFI 02087 were subjected to silica gel CC and eluted with the mixtures of petroleum ether–acetone (100:1, 50:1, 30:1, 15:1, 10:1, 5:1, 3:1), which yielded nine fractions (1–9). Fraction 7 was recrystallized with acetone to afford **6** (186.6 mg, 93.3%).

Metabolite **2**: colorless needles (acetone); mp 197–198 °C; $[\alpha]_D^{25}$ +3.46 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 217 (4.36) nm;

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