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Microbial transformation of neoandrographolide by *Mucor spinosus* (AS 3.2450)

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ABSTRACT

Microbial transformation of neoandrographolide (**1**), was performed by *Mucor spinosus* (AS 3.2450). Ten metabolites were obtained and identified as 14-deoxyandrographolide (**2**), 17,19-dihydroxy-8,13-*ent*-labdadien-16,15-olide (**3**), 3,14-dideoxyandrographolide (**4**), 7β-hydroxy-3,14-dideoxyandrographolide (**5**), 17,19-dihydroxy-7,13-*ent*-labdadien-16,15-olide (**6**), 8(17),13-*ent*-labdadien-16,15-olid-19-oic acid (**7**), 8α,17β-epoxy-3,14-dideoxyandrographolide (**8**), 8β,17,19-trihydroxy-*ent*-labd-13-en-16, 15-olide (**9**), phlogantholide-A (**10**), 19-[(β-D-glucopyranosyl)oxy]-19-oxo-*ent*-labda-8(17),13-dien-16,15-olide (**11**) by spectroscopic and chemical means. Among them, products **3**, **5**, **6**, **8** and **9** were characterized as new compounds. The inhibitory effects of compounds **1**–**11** on nitric oxide production in lipopolysaccharide-activated macrophages were evaluated and their preliminary structure–activity relationships (SAR) were discussed.

1. Introduction

Chuanxinlian is the dried aerial parts of *Andrographis paniculata* (Burm.f.) Nees, which is a Chinese herbal medicine used as an anti-inflammatory and antipyretic drug for treatment of fever, cold, laryngitis and diarrhea. The pharmacology researches show that diterpenoid lactones are the active component of *A. paniculata* (Burm.f.) Nees and possess lots of bioactive effects [1]. Neoandrographolide (Fig. 1) is one of the principal constituents of *ent*-labdane diterpenoid lactones and has many activities, such as anti-inflammatory [2–4], antiviral [5], anti-radical [6], hepatoprotective [7] and anti-human immunodeficiency virus (HIV) effects [8]. Some evidences [9,10] indicated that the *ent*-labdane diterpenoid bicyclo-skeleton and α,β-unsaturated lactone ring were the primary active structure of pyretolysis and anti-inflammatory. There were many studies on the structure modifications of andrographolide, one of the principal diterpenoids, through biological [11–13] and chemical methods [14,15], and the two derivatives, sodium 14-deoxy-12(*R*)-sulfoandrographolide (Lianbizhi) and monopotassium 14-deoxy-11,12-didehydroandrographolide-3,19-disuccinate (Chuanhuning) have been developed into antibacterial and antiviral drugs in China. However, few research of modification of neoandrographolide was reported previously.

Microbial transformation is an important tool for structure modification of organic compounds, especially natural products with complicated structures [16,17]. And this approach has some advantages over organic synthesis such as high stereo- and region-selectivity. Some transformation reactions such as hydroxylation at specific positions are difficult for chemical synthesis, but could be readily accomplished with microbial transformation [18,19]. In our previous study [20], we had reported five biotransformation products of neoandrographolide by *Aspergillus niger*. As an ongoing investigation, the present study attempts to get more types of diterpenoid derivatives by using of different fungi, and tries to find out some compounds with better activity than the substrate through pharmacological experiments.

In this work, twenty-seven kinds of fungi were screened for the bioconversion of neoandrographolide (**1**) in our experiments. Among them, *Mucor spinosus* (AS 3.2450) showed good ability to convert **1**, thus it was selected as biocatalyst for scaled-up biotransformation. Ten products were isolated from the fermentation broths. Metabolites **3**, **5**, **6**, **8** and **9** were identified as new compounds on the basis of their ^{1}H and ^{13}C NMR, DEPT, HSQC, HMBC, NOESY, and HRESIMS experiments. And the five known metabolites **2**, **4**, **7**, **10** and **11** were determined by comparison of the NMR data with those of the reported compounds [21–25]. The inhibitory effects of compounds **1**–**11** on nitric oxide production in lipopolysaccharide-activated macrophages were evaluated and their preliminary structure–activity relationships (SAR) were discussed.

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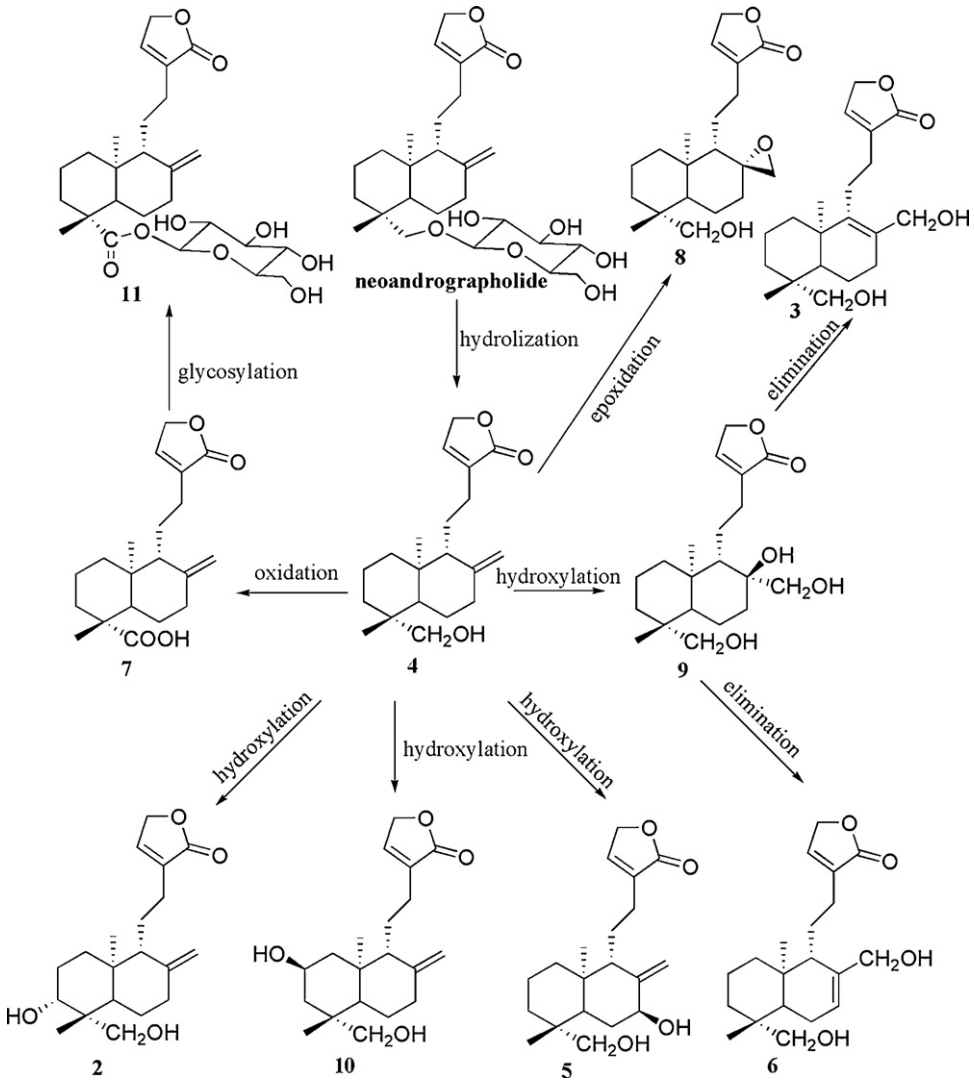

Fig. 1. Structures of products and their proposed biotransformation pathways.

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 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 Agilent 6210 TOF mass spectrometer, in m/z. Preparative HPLC separations were conducted using a Waters 600 chromatograph with an ODS column (C-18, 250 mm × 30 mm, 10 μm; YMC Co. Ltd., Japan) and Waters 490 UV detector. HPLC analyses were carried out on an ODS column (250 mm × 4.6 mm, 5 μm; YMC Co. Ltd., Japan) using a Shimadzu LC-6A liquid chromatography instrument equipped with a Shimadzu SPD-6AV UV–vis spectrometric detector. Methanol was HPLC grade (Tianjin concord technology Co. Ltd., China) and water was double distilled in our laboratory. Column chromatography was performed on silica gel (200–300 mesh) (Qingdao Marine Chemical Co. Ltd., China), Sephadex LH-20 (Pharmacia Co., Ltd., USA), and ODS (40–75 μm, Pharmacia Co., Ltd., USA). TLC was carried out on silica gel GF_{254} plate and the spots were visualized by spraying with Legal and Kedde reagents. All the analytic reagents were analytical grade and purchased from Tianjin DaMao Chemical Company (Tianjin, China).

2.2. Substrate

Neoandrographolide (>98%) was isolated from the aerial parts of *A. paniculata* (Burm.f.) Nees by ourselves, and was characterized by comparison of the NMR data with the reference.

2.3. Microorganisms

M. spinosus (AS 3.2450) was purchased from China General Microbiological Culture Collection Centre.

2.4. Medium

All culture and biotransformation experiments were performed in potato medium as following procedure: 200 g of mincing 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.