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Microbial transformation of 14-deoxy-11, 12-didehydroandrographolide and 14-deoxyandrographolide and inhibitory effects on nitric oxide production of the transformation products

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ABSTRACT

Microbial transformations of 14-deoxy-11, 12-didehydroandrographolide (**a**) and 14-deoxyandrographolide (**b**) were performed by *Cunninghamella blakesleana* (AS 3.970), respectively. Sixteen metabolites were obtained and their structures were elucidated by spectroscopic data analyses. Among these metabolites, 3α, 12*S*, 19-trihydroxy-8(17), 9(11)-*ent*-labdadien-16, 15-olide (**a7**), 3-oxo-8α, 17β-epoxy-14-deoxyandrographolide (**b2**), 3α, 17, 19-trihydroxy-8, 13-*ent*-labdadien-16, 15-olide (**b6**), and 9β-hydroxy-14-deoxyandrographolide (**b9**) are new compounds. The configuration of C-12 in metabolite **a7** was determined as *S* by GIAO method. The proposed metabolic pathways of 14-deoxy-11, 12-didehydroandrographolide and 14-deoxyandrographolide by *C. blakesleana* were drawn. The inhibitory effects of these compounds on nitric oxide production in lipopolysaccaride-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 famous traditional Chinese and Ayurvedic medicine used as an anti-inflammatory and antipyretic drug for treatment of fever, cold, laryngitis and diarrhea. The pharmacological researches show that diterpenoid lactones are major active components of *A. paniculata* [1]. 14-Deoxy-11, 12-didehydroandrographolide and 14-deoxyandrographolide (Figs. 1 and 2), two principal *ent*-labdane diterpenoid lactones of *A. paniculata*, exhibit anti-inflammatory [2], anti-hypertensive [3], immunoregulatory [4], and anti-tumor [5] properties.

Nitric oxide (NO) is an important cellular messenger molecule involved in many physiological and pathological processes within the mammalian body both beneficial and detrimental [6]. The chronic expression of NO is associated with various inflammatory conditions including multiple sclerosis, arthritis and ulcerative colitis [7]. Therefore, the *in vitro* inhibitory effects of diterpenoid lactones on NO production can provide scientific supports for the traditional use of the aerial parts of *A. paniculata* as a remedy for various inflammatory conditions.

Microbial transformation provides an important tool for structure modification of organic compounds, especially natural products with potent biological activities [8,9]. This method is known for its high stereo- and region-selectivity, ease of handling, low cost, and environmental-friendly nature [10,11].

Microbial transformation was first applied to the structural modification of neoandrographolide by our group [12,13]. Then, biotransformation of other principle diterpenoids of *A. paniculata* and cytotoxicity of the transformed products were studied by several other groups [14–18]. As our continuous investigation, the present work attempts to get more diterpenoid analogues by microbial biotransformation using different fungi in order to assess the structure-activity relationships of this class of constituents and tries to find some compounds with better anti-inflammatory activity.

This article describes the bioconversion of 14-deoxy-11, 12-didehydroandrographolide (**a**) and 14-deoxyandrographolide (**b**) by *Cunninghamella blakesleana* (AS 3.970). Sixteen bioconversion products, including four new compounds were isolated

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and identified. In addition, the inhibitory effects of these compounds on nitric oxide production in lipopolysaccaride-activated macrophages were 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 Agilent 6210 TOF mass spectrometer, in m/z. Melting points were determined with an X-5 hot stage microscope melting point apparatus (uncorrected). 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 (C-18, 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) 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. Substrates

Both 14-deoxy-11, 12-didehydroandrographolide and 14-deoxyandrographolide (>98%) were isolated from the aerial parts of *A. paniculata* (Burm.f.) Nees by ourselves, and were characterized by comparison of the NMR data with the references.

2.3. Microorganism

C. blakesleana (AS 3.970) 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 minced husked

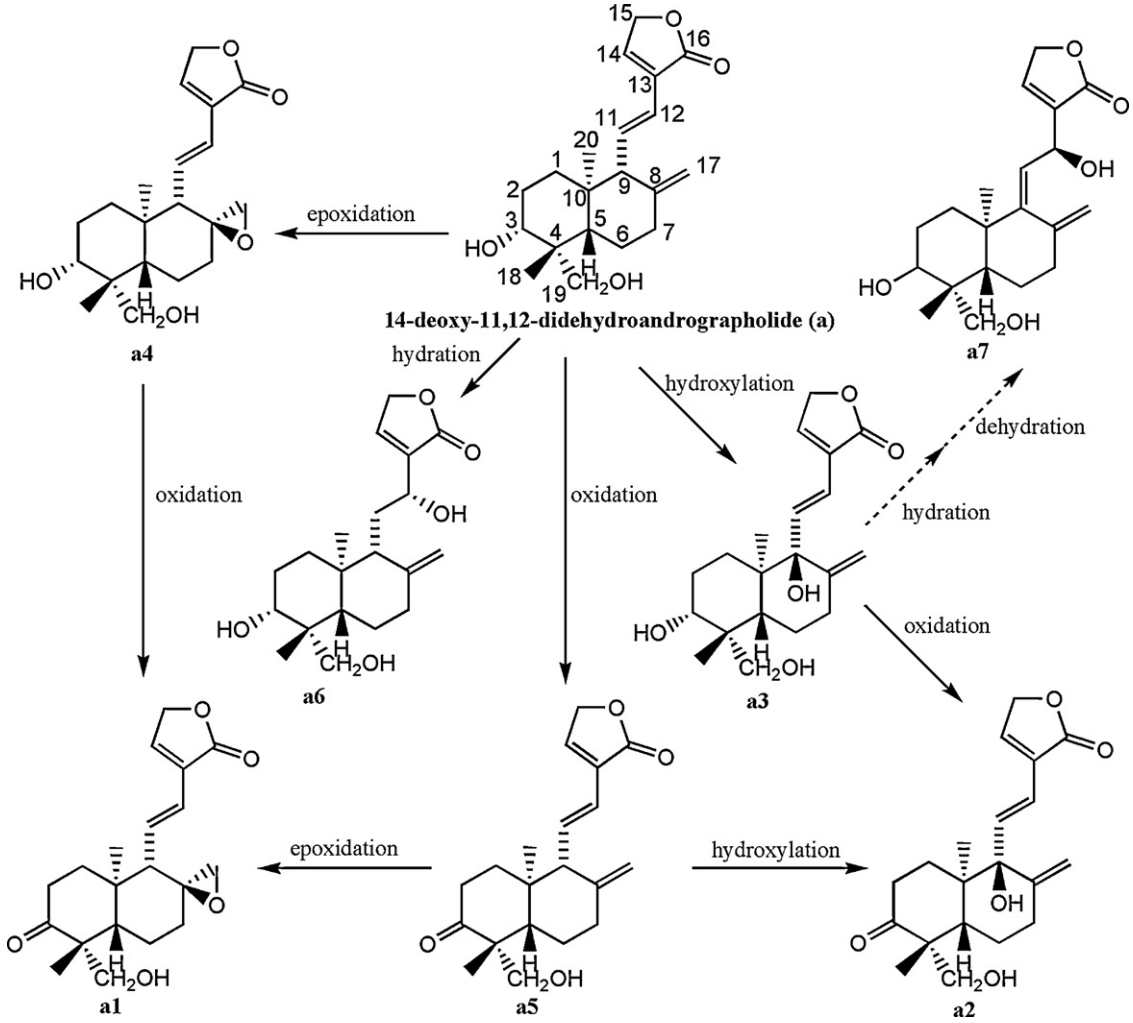

Fig. 1. Structures of biotransformed products of 14-deoxy-11, 12-didehydroandrographolide (**a**) and their proposed metabolic pathway.