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ScienceDirect
*Chinese Journal of Natural Medicines* 2016, **14**(10): 0783−0788
**Chinese Journal of Natural Medicines**

# Diversity synthesis of tetrahydroprotoberberines glycosides by combined chemical and microbial catalysis

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Available online 20 Oct., 2016

**[ABSTRACT]** The present study was designed to construct the structurally diverse library of tetrahydroprotoberberines (THPBs) by combining the methods of chemical nonselective demethylation and microbial glycosylation. HPLC-MS/MS analyses tentatively identified 12 de-methylated and 9 glycosylated derivates of THPBs and 5 rarely oxidized glycosides of THPBs in the library. Through this effort, we achieved not only a variety of the THPBs and their glycosides but also tested the catalytic characteristics and capabilities of *G. deliquescens* NRRL 1086.

**[KEY WORDS]** THPBs library; Diversity synthesis; Microbial glycosylation; Chemical demethylation

**[CLC Number]** R914 **[Document code]** A **[Article ID]** 2095-6975(2016)10-0783-06

## Introduction

Tetrahydroprotoberberines (THPBs), a large class of naturally occurring alkaloids from the traditional Chinese herb "*Corydalis* and *Stephania*", have been demonstrated to possess various biological activities, such as analgesic, anticonvulsant, antianxiety, antidepressant, and antipsychotic activities [1]. THPBs are characterized by a tetracyclic benzyl isoquinoline skeleton and their most common structures in nature are that methoxyl or hydroxyl groups substituent at the C-2, C-3 and C-9, C-10 positions on the A and D aromatic rings, respectively [2], which limit their structural diversities and restrict their further applications in the research and development of new drugs based on these core structures.

Over the past decades, the importance of carbohydrates in the life sciences has been increasingly recognized, alongside with advances in glycochemistry and glycobiology. Glycosylations of small molecules are critical for their functions [3–4]. A significant number of glycosylated small molecules, such as heparin, amikacin, and cytarabine, have been shown to be clinically useful for the treatment of bacterial and fungal infections, cancer, and other diseases [5–6]. Although glycosides are abundant in nature, the glycosidic alkaloids, especially the THPBs glycosides, are very rare [7]. In our previous work [8–9], we have tentatively investigated glycosylation of THPBs by *Gliocladium deliquescens* NRRL1086 and found some interesting results such as the selectivity on phenolic hydroxyl on C-9, and the enhanced tissue factor inhibitory activities, compared with their aglycone [10]. However, due to the limited substrates and the chemical characteristics of THPBs, we have failed to synthesize more glycosides of THPBs or obtain more properties of this biotransformation culture.

Structurally diversity synthesis is a new synthetic strategy to populate new chemical space with novel drug-like compounds containing a high degree of molecular diversity and biological relevancy; and one-pot multistep approaches

**[Received on]** 27- Nov.-2015
**[Research funding]** This work was funded by the National Nature Science Foundation of China (No. 21302052) and the Program for New Century Excellent Talents in University. Thanks also give to the '111 Project' from the Ministry of Education of China, and the Fundamental Research Funds for the Central Universities (No. JKZ2011017).
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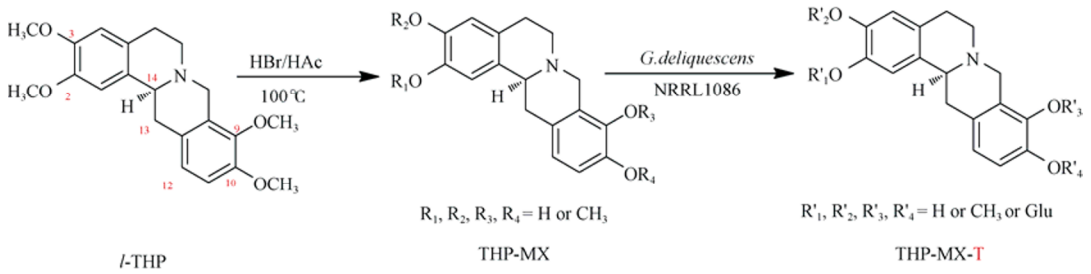
These authors have no conflict of interest to declare.

do not require intermediate work-up and purification steps and thus expedite synthesis [11]. Our laboratory has been actively engaged in the synthesis of molecules with biological activities using green conditions, particularly in the synthesis of complex natural glycoconjugates by microbial biotransformation [8–9, 12]. As a result, we have begun a program to explore the synthesis of the structural diversity of THPBs (*Scheme* 1). As a first step toward this goal we have chosen the hydrobromic acid as an non-selective demethylation agent, which can introduce the potential glycodic linkage sites of the skelton, then we have subjected the mixture to culture of *Gliocladium deliquescens* NRRL1086 for microbial glycosylation; with this combination it will not only construct a THPBs library containing unusual glycosides of THPBs, but also further explore the substrates scope and catalytic capability of *G. deliquescens* NRRL 1086.




**Scheme 1 The synthesis strategy of THPBs glycosides**

## Materials and Methods

*Instrumentations and general procedures*

A reversed-phase ODS-2 column (Hedera; 5-μm, 250 mm × 4.6 mm, Hanbon Sci. & Tech., Jiangsu, China) was used for all chromatographic separations. A linear solvent gradient of solvents A (0.5% aqueous acetic acid and 20 mmol·$L^{-1}$ ammonium acetate) and B (acetonitrile) was used as follows: 5%− 6% B at 0−10 min, 6%−7% B at 10−20min, 7%−8% B at 20−25min, 8%−10% B at 25−45min, 10%−11% B at 45−55 min, 11%−12% B at 55−75 min, 12%−18% B at 75−85 min, 18%−23% B at 85−100 min, and 23%−80% B at 100−120 min, and the detection wavelength was set at 285 nm.

*Gliocladium deliquescens* NRRL1086 was obtained from a courtesy of Prof. J. P. N. Rosazza of University of Iowa, Iowa State, USA. *L*-tetrahydropalmatine (*L*-THP) was obtained commercially from Aladdin Co. and the purity was determined to be higher than 99% by normalization of the peak areas detected by HPLC.

*Preparation of the mixed substrates (THP-MX)* 2 g of *L*-THP was dissolved in 15 mL of 40% hydrobromic acid and 10 mL of acetic acid. After stirring for 2 h at 100 ºC, 10 mL of water was added and the reaction mixture was cooled to room temperature. The white solid was filtered and the pH of the residual mixture was adjusted to pH 8 by using saturated $NaHCO_3$ solution and extracted with ethyl acetate. The extract was evaporated and dried to give the mixed substrates (THP-MX).

*Preparation of the transformation products (THP-MX-T)* The mixed substrates (THP-MX) were transformed by the *Gliocladium deliquescens* NRRL1086 using the following procedures: Cultures were grown by a two-stage procedure in 30 mL of potato medium (PD) held in 150-mL culture flasks. The PD medium was prepared as follows: 200 g of peeled potatoes were cut into pieces, boiled in water for 20 min, and filtered. Twenty grams of glucose, 3 g of $KH_2PO_4$, and 1.5 g of $MgSO_4·7H_2O$ were added into the filtrate that was further diluted with distilled water to 1 L and autoclaved at 121 ºC for 15 min before use. Cultures were incubated with shaking at 180 r·$min^{-1}$ at 28 ºC on rotary shakers. One milliliter of *G. deliquescens* NRRL1086 inoculum derived from 24-h-old stage I cultures was used to initiate stage II cultures, which were incubated for 24 h before adding 10 mg of the mixed substrates (THP-MX) in 1 mL of acetone, and incubations were conducted as above. Substrate controls were consisted of sterile medium and substrate and incubated under the same conditions but without microorganism. The cultures were filtered and extracted with ethyl acetate at 120 h after addition of substrates. The organic solvent layer was removed and evaporated to give the transformation products (THP-MX-T).

*LC-MS analysis*

The mixed substrates (THP-MX) and their transformation products (THP-MX-T) were dissolved in methanol and analyzed by LC-MS/MS.

The LC/MS-IT-TOF (Shimadzu, Tokyo, Japan) mass operation parameters were set as follows: ion electrospray, nebulizing gas ($N_2$) flow rate 1.5 L·$min^{-1}$, drying gas ($N_2$) pressure 0.1 MPa, applied probe voltage +4.5 and −3.5 kV, CDL voltage set at constant mode (optimized by autotuning), and CDL temperature 200 ºC. Mass spectrometry was conducted in the full scan and automatic multiple-stage fragmentation scan modes over an *m/z* range of 100−800 for MS, $MS^2$, and $MS^3$ scan. The ion accumulation time was set at 30 ms. Argon was used as the collision gas, and collision energy were set at 30% and 50% for $MS^2$ and $MS^3$ respectively to induce fragmentations. Trifluoroacetic acid (TFA) sodium solution was used as the standard.

## Results and Discussion

Based on the reported literature [13–16] and a detailed MS analy-