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Preparative separation of four major alkaloids from medicinal plant of *Tripterygium Wilfordii Hook F* using high-speed counter-current chromatography

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Abstract

A high-speed counter-current chromatography (HSCCC) method was developed for preparative isolation and purification of alkaloids from *Tripterygium Wilfordii Hook F*. Preparative HSCCC with a two-phase solvent system composed of petroleum ether–ethyl acetate–ethanol–water (6:4:5:8, v/v/v/v). The organic phase was used as the stationary phase of HSCCC, and the aqueous phase as the mobile phase. Seven hundred milligrams total alkaloids yielded 210 mg of wilfortrine, 90 mg of wilfordine, 220 mg of wilforgine and 100 mg of wilforine, with the purity of 90.3%, 92%, 99.5% and 93.5% in one step separation, respectively.

Keywords: *Tripterygium Wilfordii Hook F*; Preparative separation; Alkaloid; HSCCC

1. Introduction

Tripterygium Wilfordii Hook F (TWHF) is a traditional Chinese herb grown in the south of China and used for various immune and inflammatory diseases in China [1]. The extracts of the roots of TWHF by ethanol, ethyl acetate and other solvents have potent immunosuppressive, anti-cancer and anti-inflammatory properties. The diverse extracts are used widely in China for the treatment of a number of autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus, and skin diseases [2–6].

Pharmacological properties of TWHF were studied extensively over the last years, and the results obtained could explain the traditional use of the plant and confirm its efficacy. The main active constituents of the herb have been reported to be diterpenes, triterpenes and alkaloid compounds [7,8]. Wilfortrine, wilfordine, wilforgine and wilforine (the chemical structures shown in Fig. 1) were originally separated from TWHF [9]. Wilfortrine and wilforine have been reported to possess immunosuppresive effects, wilforine is effective in treatment of rheumatoid arthritis [10]. Wilfortrine can inhibit leukemia cell growth in mice [11,12], and show anti-HIV activity [13].

So far, wilfortrine, wilfordine, wilforgine and wilforine have been purified from TWHF by several steps, including chromatography and crystallization. However, those conventional methods may encounter various problems. For example, the compound of interest is often strongly adsorbed onto the solid support of conventional silica gel column chromatography, which result in low recoveries. Existing HPLC methods are not suitable for large-scale isolation of wilfortrine, wilfordine, wilforgine and wilforine. Further studies on pharmacological and clinical effects of wilfortrine, wilfordine, wilforgine and wilforine necessitate the development of an efficient preparative separation method of these compounds. Such a method will also facilitate quality control and improvement of the quality of existing TWHF products.

High-speed counter-current chromatography (HSCCC), first invented by Ito [14], is a support-free liquid–liquid partition chromatographic technique, and eliminates irreversible adsorption of the sample onto the solid support [15]. With a large amount of sample injection, multiform relative pure substances can be obtained at one step in large amount. It is especially suitable for separation and purification of active components

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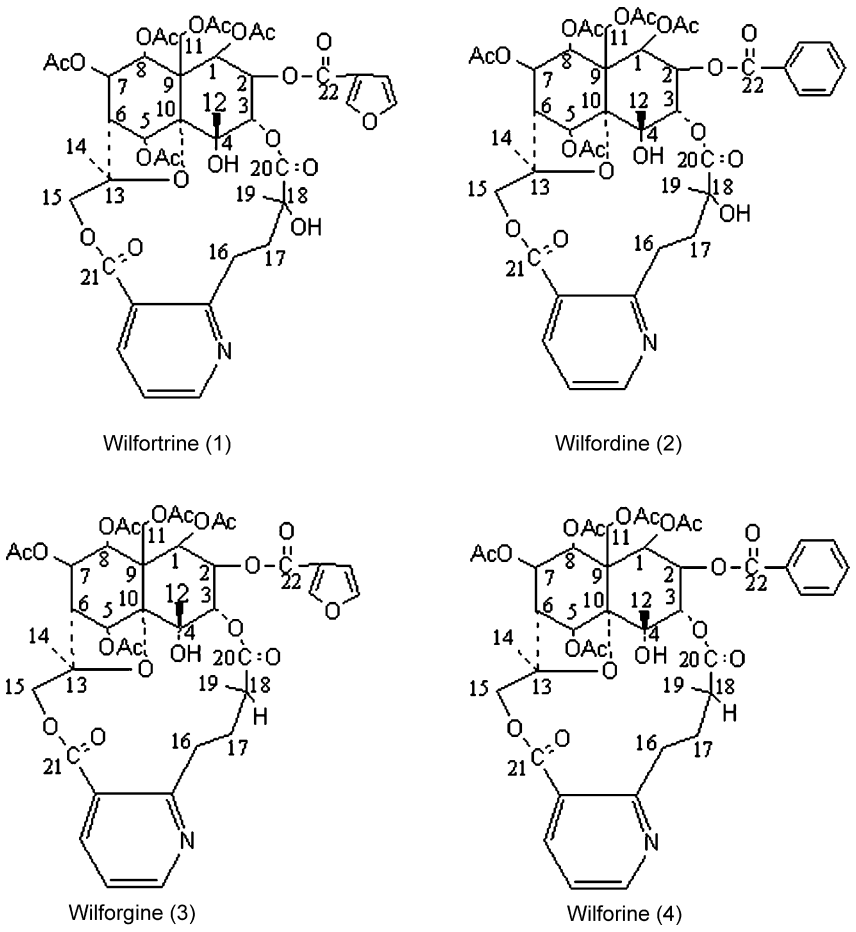

Fig. 1. Structure of alkaloids **1**–**4** isolated from *Tripterygium Wilfordii Hook F*.

from natural products [16–22]. However, no report has been published on the use of HSCCC for the isolation and purification of wilfortrine, wilfordine, wilforgine and wilforine from TWHF.

The present paper describes the successful preparative separation and purification of wilfortrine (component **1**), wilfordine (component **2**), wilforgine (component **3**) and wilforine (component **4**) from the partially purified total alkaloids from TWHF by HSCCC.

2. Experimental

2.1. Reagents and materials

Acetonitrile (MeCN) was HPLC grade. Deionized water was prepared by a Milli-Q water purification system from Millipore (Molsheim, France). The other regents were of analytical grade. The root bark of TWHF was obtained from DND Pharmaceutical Co. Ltd. (XinChang, Zhejiang province, China). Wilfortrine, wilfordine, wilforgine and wilforine standards (purity >95%, determined by HPLC) were obtained in our lab by column chromatography, identity of the isolated compounds were confirmed by spectral (MS, ^{1}H NMR and ^{13}C NMR) methods.

2.2. Apparatus

HSCCC was performed using a Model TBE-1000A HSCCC system manufactured by Tauto Biotech Co. Ltd., Shanghai, China, equipped with a 1000 ml coil column made of polytetrafluoroethylene tubing (3.0 mm). The β-value of the preparative column varied from 0.59 at the internal layer to 0.75 at the external layer ($\beta = r/R$, where r is the distance from the coil to the holder shaft, and R is the revolution radius or the distance between the holder axis and the central axis of centrifuge). The revolution speed of the apparatus could be adjusted in a range between 0 and 600 rpm. The solvent was pumped into the column by a Model SD-9002 constant-flow pump (Beijing Shengyitong Technology Development Co. Ltd.), and continuously delivered by 254 nm absorption with a Model 8823B UV detector (Beijing Institute of New Technology Application), the data was displayed and analyzed simultaneously on a Model N2010 workstation (Zhejiang University, Hangzhou, China). The experimental temperature was adjusted by HX-2050 constant temperature circulating implement (Beijing Boyikang Lab Implement, Beijing, China). A manual injection valve with an 80 ml loop was used to introduce the sample into the column.

The HPLC equipment used was an Agilent 1100 system, consisting of a quaternary pump (G1311A), a column ther-