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Characterization and simultaneous determination of immunosuppressive decalins in red yeast rice by ultra-high-performance liquid chromatography hyphenated with mass spectrometry

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ABSTRACT

Decalins are secondary metabolites of red yeast rice with immunosuppressive effects on human T cell proliferation. In this study, ultra-high-performance liquid chromatography hyphenated with quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF-MS) was employed for elucidation of the mass fragmentation patterns of decalins by collision-induced dissociation tandem mass spectrometry (CID-MS/MS). Based on the MS/MS fragmentation patterns of the authentic decalin standards as well as high mass accuracy, a new decalin in the crude extract of red yeast rice was further putatively identified. Moreover, a quantitative analysis method of five immunosuppressive decalins by ultra-high-performance liquid chromatography hyphenated with triple quadrupole tandem mass spectrometry (UHPLC–QQQ-MS) under multiple reaction monitoring (MRM) mode was developed and validated. This method exhibits limits of detection from 0.44 to 1.96 mg/kg, and precision variations were less than 3.2%, and the recovery was in the range of 82 and 105% with RSD less than 5.4%. This method was successfully applied in the quantitative analysis of decalins in different types of red yeast rice. The results showed that decalins only exist in functional red yeast rice but not in the common one. The study demonstrated that UHPLC-Q-TOF-MS and UHPLC–QQQ-MS methods described in this paper are powerful and reliable tools for the quality control of red yeast rice.

1. Introduction

Red yeast rice, produced from the fermentation of steamed rice using the fungus *Monascus purpureus* [1], has been used as food and traditional medicine for centuries in China [2]. It consists mainly of secondary metabolic products of the fermentation including pigments, monacolins, decalins, γ-aminobutyric acid (GABA) and unsaturated fatty acids [3–7].

Recently, much attention has been paid to the secondary metabolic products of red yeast rice since the discovery of monacolins, which are potent inhibitors of HMG-CoA reductase and can lower blood lipid levels in both animal models and humans [8]. Monacolin K, also known as lovastatin, the major monacolin in red yeast rice, has been chosen as a marker compound for the quality control of red yeast rice in Chinese official monograph [2]. Decalins, another type of secondary metabolic products of red yeast rice, have close biosynthetic relationship with monacolins. The chemical structures of decalins are very similar to monacolins, with the only difference that the lactone part in monacolin is replaced by carboxyl acid group in decalin skeleton. Biogenetically, decalins are presumed to be formed by β-oxidation and dehydrogenation from monacolins [9,10]. Two decalin compounds (monascusic acid A and heptaketide) were isolated from red yeast rice but their biological activities were not investigated [11]. Recently, we reported the extraction, purification and characterization of a series of decalins with much chemical diversity in red yeast rice, including five new decalins. All of them were found to be capable of suppressing human T cell proliferation in a dose-dependent manner [12].

Ultra-high-performance liquid chromatography (UHPLC) hyphenated with mass spectrometry has been proven to be a powerful tool for chemical analysis since it allows efficient chromatographic separation and accurate identification of individual

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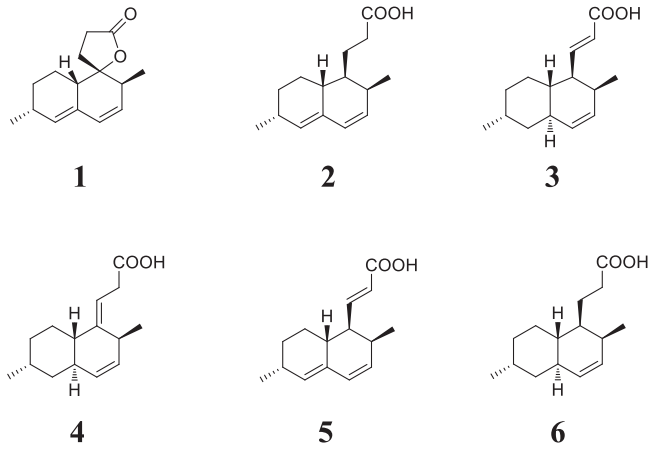

Fig. 1. Chemical structures of six decalins isolated from red yeast rice (**1**: monascusic lactone A; **2**: monascusic acid A; **3**: monascusic acid B; **4**: monascusic acid C; **5**: monascusic acid D; **6**: heptaketide).

compounds [13–15]. The mass fragmentation by collision-induced dissociation tandem mass spectrometry (CID-MS/MS) has been widely used to provide valuable structural information for the targeted components in the crude extract. Of particular interest is the identification of novel components in the herb extract based on time-of-flight (TOF) mass spectrometric studies of their analogs [16], because this online identification of specific category of compounds could also facilitate the focused isolation and purification in subsequent experiment. Regarding the detection method of mass techniques, multiple reaction monitoring (MRM) mode has become the preferred method for the quantitative analysis using triple quadrupole mass spectrometry [17,18] due to its extraordinary detection sensitivity.

Although our previous research have demonstrated that decalins are a category of bioactive compounds with chemical diversity and considerable amounts in red yeast rice, no report on their quantitative analysis and distributions in red yeast rice were disclosed by now. Therefore in this study, the mass fragmentation patterns of six decalins isolated by us from red yeast rice were comprehensively analyzed in CID-MS/MS experiments. By the established methods of spectrum interpretation and MS/MS fragmentation pattern of decalins, a new decalin in the crude extract of red yeast rice was discovered. Furthermore, the major decalins in different types of red yeast rice were simultaneously quantified by ultra-high-performance liquid chromatography hyphenated with electrospray ionization triple quadrupole tandem mass spectrometry.

2. Experimental

2.1. Reagents and chemicals

HPLC grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). HPLC-grade formic acid was purchased from Fluka (Buchs, Switzerland). Water was purified with a Milli-Q Plus system (Millipore, Inc., MA, USA) at 18.2 MΩ cm.

Six authentic standards, namely monascusic lactone A (**1**), monascusic acid A (**2**), monascusic acid B (**3**), monascusic acid C (**4**), monascusic acid D (**5**) and heptaketide (**6**) (Fig. 1) were isolated from red yeast rice in our laboratory [12] and were identified by IR, UV, NMR spectroscopic analysis (purity >98%, HPLC). Each accurately weighed standard was dissolved in methanol to give individual stock solutions. A series of calibration curve solutions were prepared by appropriate dilution of the stock solution with blank matrix solution in order to prepare calibrators. All solutions were stored at 4 °C in refrigerator before analysis.

2.2. Sample preparation

A total of ten samples of red yeast rice were collected from different provinces in China, namely: four batches of common red yeast rice encoded as CR-1, CR-2, CR-3 and CR-4 were purchased from Fujian, Zhejiang, Sichuan and Yunnan province, respectively; Two batches of functional red yeast rice were purchased from Zhejiang and Jiangxi province, encoded as FR-1 and FR-2 respectively. Besides, two batches of functional red yeast rice in the form of capsule formulations, namely Xuezhikang (Beijing Peking University WBL Biotech Co., Ltd) encoded as FR-3 and Hongqu Xiaozhisu (Beijing Jiying Biotech Co., Ltd) encoded as FR-4 were purchased accordingly, whereas two batches of Zhibituo tablets encoded as FR-5 and FR-6 were purchased from Chengdu Di'ao Pharmaceutical Ltd. and Yunnan Yong'an Pharmaceutical Ltd, respectively. Voucher specimens were deposited in School of Chinese Medicine, Hong Kong Baptist University.

Powdered sample (0.2 g) was accurately weighed into a 15 mL centrifugal tube and 5 mL methanol was added. The mixture was sonicated for 30 min with occasional shaking and then centrifuged at 1800 × *g* for 5 min. The sample was further extracted with 4 mL methanol. The supernatant was combined into a 10 mL volumetric flask and then made up to the mark with methanol. The sample solution was filtered through a 0.22 μm PTFE filter and transferred into vials for injection.

2.3. UHPLC-Q-TOF-MS system

2.3.1. Chromatographic analysis

UHPLC was performed on Waters Acquity™ ultra-high-performance liquid chromatography (UHPLC) system (Waters Corp., Milford, USA). The chromatography was performed on an ACQUITY UHPLC BEH C_{18} column (1.7 μm, 100 mm × 2.1 mm i.d., Waters). The mobile phase consisted of solvent A (0.1%, v/v, of formic acid in water) and solvent B (0.1%, v/v, of formic acid in acetonitrile). A gradient elution procedure was used: 0–12 min, 20–80% B; 12–14 min, 80–100% B; 14–16 min, 100% B. The flow rate was 0.35 mL/min, the injection volume was 5 μL, and the column temperature was maintained at 40 °C.

2.3.2. Mass spectrometry

Mass spectrometry was performed on a Bruker MicrOTOFQ system with an electrospray ionization (ESI) interface (Bruker Daltonics, Bremen, Germany) operating in positive mode. The MicrOTOFQ source parameters were as follows: end plate offset, −500 V; capillary voltage, 4500 V; collision energy, 7 eV; nebulizing gas (N_2) pressure, 2.0 bar; drying gas (N_2) flow rate, 8.0 L/min; drying gas temperature, 180 °C; Mass range, *m/z* 50–1500.

The MS/MS experiments were carried out by setting the Q-TOF Premier quardrupole to allow ions of interest to pass prior to fragmentation in the collision cell with collision energies varying between 15 and 20 eV.

2.4. Quantification method

Quantification of the decalins was carried out on an ultra-high-performance liquid chromatography hyphenated with triple quadrupole tandem mass spectrometry (UHPLC–QQQ-MS).

Chromatographic separations of decalins were performed on a Agilent 1290 Infinity ultra-high-performance liquid chromatography system (Agilent) using an ACQUITY UHPLC BEH C_{18} column (1.7 μm, 100 mm × 2.1 mm i.d., Waters). A gradient program was used with mobile phase consisting of solvent A (0.1%, v/v, of formic