

DOCTORAL THESIS

Chemical studies on the active components in red yeast rice

Zhu, Lin

Date of Award:
2012

[Link to publication](#)

General rights

Copyright and intellectual property rights for the publications made accessible in HKBU Scholars are retained by the authors and/or other copyright owners. In addition to the restrictions prescribed by the Copyright Ordinance of Hong Kong, all users and readers must also observe the following terms of use:

- Users may download and print one copy of any publication from HKBU Scholars for the purpose of private study or research
- Users cannot further distribute the material or use it for any profit-making activity or commercial gain
- To share publications in HKBU Scholars with others, users are welcome to freely distribute the permanent URL assigned to the publication

**Chemical Studies on the Active Components in
Red Yeast Rice**

ZHU Lin

**A thesis submitted in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy**

Principal Supervisor: Prof. HSIAO Wen Luan

Hong Kong Baptist University

August 2012

ABSTRACT

A
T
-
-
-
I
I
I
I

Red yeast rice, produced from the fermentation of steamed rice using the fungus *Monascus purpureus*, has been applied as food colorant, dietary material and folk medicine in Asian countries for thousands of years. Red yeast rice possesses many pharmacological activities like lipid-lowering effect, anti-cancer effect, anti-inflammatory effect, management of cardiovascular disease and so on, which are ascribable to the coexistence of many different types of components. Red yeast rice consists mainly of rice and secondary metabolites of the fermentation. *Monascus* can produce several kinds of secondary metabolites including pigments, monacolins, γ -aminobutyric acid (GABA), and unsaturated fatty acids. However, there are still additional secondary metabolites from red yeast rice remained unidentified, thus their contribution to the biological activities of red yeast rice are unknown.

I
I
I
I

In order to clarify the underlying chemical foundation and evidence for the therapeutic effects of red yeast rice, detailed chemical analysis of red yeast rice was performed in our study. The aim of our study was focused on rapid characterization, isolation and structural elucidation of novel compounds from red yeast rice, as well as their biological activities screening. To achieve this goal, UHPLC-MS technique was utilized for pre-targeting. Isolation and purification of the desired compounds were subsequently carried out by using chromatographic methods. The purified compounds were structurally elucidated using MS and NMR techniques and chemical transformation. Finally, the bioassay of the activities of the isolated novel compounds was conducted. The results obtained from the entire research are summarized as below:

- (1) Two new dehydromonacolins, together with nine known monacolins, were isolated from the ethyl acetate extract of red yeast rice. The structures of the two new dehydromonacolins elucidated on the basis of MS and NMR spectroscopic evidence were further confirmed by semisynthesis. Dehydromonacolin N, possessing a C2 side chain, is unprecedented in the natural monacolin family. The cytotoxicity assay of dehydromonacolins disclosed the structure-activity relationship which shows that dehydromonacolins with extended alkyl side chain exerted more potent cytotoxic effects toward cancer cells. This is a novel discovery for the biological activities of dehydromonacolins.
- (2) Seven decalins including five new compounds were isolated from the ethyl acetate extract of red yeast rice. Their structures were elucidated by means of NMR and mass spectroscopic analyses. Monascus lactone A is the first reported naturally occurring decalin derivative possessing a spiro lactone at C-1 position. The immunosuppressive effects of these decalins on human T cell proliferation were investigated in this study. All of them could suppress human T cell proliferation in a dose-dependent manner. This is the first report on the immunosuppressive activity of decalins.

(3) Ultra high performance liquid chromatography hyphenated with quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF-MS) was employed for characterization of the mass fragmentation patterns of the isolated decalins by collision-induced dissociation tandem mass spectrometry (CID-MS/MS). From the MS/MS fragmentation patterns of the authentic standards as well as high mass accuracy, two new decalins in the crude extract of red yeast rice were further putatively identified. A quantitative analysis method of 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. The method exhibited limits of detection from 0.44 to 1.96 mg/kg, and precision and repeatability variations were less than 2.8%, and the recovery was in the range of 77% and 105% with RSD less than 9.2%. This method was successfully applied for the analysis of decalins in different types of red yeast rice. The results showed that decalins only existed in functional red yeast rice but not in the common red yeast rice. Heptaketide was proposed to be another marker for the quality control of functional red yeast rice.

In conclusion, this research demonstrated the rapid and effective isolation of series of desired monacolins and decalins by the pre-targeting method using LC-MS. As a result, the novel chemical structures and innovative bioactivities of the isolated compounds definitely contributed to both chemical and biological diversities of red yeast rice.

TABLE OF CONTENTS

DECLARATION.....	i
ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	iv
TABLE OF CONTENTS.....	vi
LIST OF FIGURES.....	xii
LIST OF TABLES.....	xvi
LIST OF SCHEMES.....	xviii
LIST OF ABBREVIATIONS.....	xix
 CHAPTER 1	
INTRODUCTION AND DESIGN OF THE RESEARCH.....	1
1.1 GENERAL INTRODUCTION OF RED YEAST RICE (“HONG QU”).....	2
1.2 PRODUCTION OF RED YEAST RICE (“HONG QU”).....	3
1.2.1 Solid-state Fermentation.....	5
1.2.2 Liquid/Submerged Cultivation.....	7
1.3 PHARMACOLOGICAL ACTIVITIES OF RED YEAST RICE (“HONG QU”).....	9
1.3.1 Hypolipidemic Effect.....	9
1.3.2 Anti-cancer Effect.....	13
1.3.3 Anti-inflammatory Effect.....	15
1.3.4 Management of Cardiovascular Disease.....	16
1.3.5 Anti-osteoporosis Effect.....	19

1.3.6 Anti-diabetic Effect.....	20
1.3.7 Antioxidant Effect.....	21
1.3.8 Anti-hypertensive Effect.....	23
1.3.9 Anti-Alzheimer's Effect.....	24
1.4 CHEMICAL CONSTITUENTS AND THEIR BIOLOGICAL ACTIVITIES	
OF RED YEAST RICE (“HONG QU”).....	25
1.4.1 Monacolins (Statins).....	25
1.4.1.1 History of Monacolins (Statins).....	25
1.4.1.2 Monacolins (Statins) in Red Yeast Rice.....	30
1.4.1.3 Lipid-lowering Effect of Statins.....	33
1.4.1.4 Other Effects.....	37
1.4.1.5 Side Effects.....	41
1.4.2 Pigments.....	46
1.4.2.1 Chemical Structures and Biosynthesis.....	46
1.4.2.2 Stability.....	49
1.4.2.3 Biological Activities.....	49
1.4.3 Citrinin.....	50
1.4.3.1 General Information.....	50
1.4.3.2 Toxicity.....	51
1.4.3.3 Biosynthesis Pathway.....	51
1.4.3.4 Restriction.....	52
1.4.3.5 Stability.....	52
1.4.4 Organic Acids.....	53
1.5 QUALITY CONTROL AND SAFETY	55
1.6 AIMS, OBJECTIVES AND DESIGN OF THE STUDY.....	55

1.6.1 Aims and Objectives.....	55
1.6.2 Design of the Study.....	57
1.6.2.1 Preliminary Targeting of the Statins and Analogues in Representative Red Yeast Rice	57
1.6.2.2 Isolation and Purification of the Target Compounds.....	58
1.6.2.3 Structural Elucidation of the Isolated Compounds.....	58
1.6.2.4 Biological Activities Screening.....	58
1.6.2.5 Mass Fragmentation Pattern Study and Quantitation of the Isolated Novel Compounds in Red Yeast Rice.....	58
1.7 STRUCTURE OF THE THESIS.....	59

CHAPTER 2

ISOLATION, STRUCTURAL ELUCIDATION AND CYTOTOXICITY

EVALUATION OF MONACOLINS.....	60
2.1 INTRODUCTION.....	60
2.2 EXPERIMENTAL.....	62
2.2.1 General Experimental	62
2.2.2 Reagents.....	63
2.2.3 Material.....	63
2.2.4 Pre-targeting Criteria for Statin.....	63
2.2.5 Extraction and Isolation.....	65
2.2.6 Synthesis of Compound 1.....	70
2.2.7 Synthesis of Compound 3.....	71
2.2.8 Cell Culture and Cytotoxicity Assay of Compounds 1-3, 8, and 11.....	71
2.3 RESULTS AND DISCUSSION.....	72

2.4 CONCLUSIONS.....	85
----------------------	----

CHAPTER 3

ISOLATION, STRUCTURAL ELUCIDATION AND

IMMUNOSUPPRESSIVE ACTIVITY OF DECALINS.....	87
--	-----------

3.1 INTRODUCTION.....	87
-----------------------	----

3.2 EXPERIMENTAL.....	88
-----------------------	----

3.2.1 General Experimental Procedures.....	88
--	----

3.2.2 Materials.....	88
----------------------	----

3.2.3 Extraction and Isolation.....	89
-------------------------------------	----

3.2.4 Cell Culture.....	92
-------------------------	----

3.2.5 Examination of the Effects of Compounds 13-19 on Human T Cell	
---	--

Proliferation.....	93
--------------------	----

3.2.6 Cytotoxicity of Compounds 13-19 on Human T lymphocytes.....	94
---	----

3.3 RESULTS AND DISCUSSION.....	94
---------------------------------	----

3.4 CONCLUSIONS.....	114
----------------------	-----

CHAPTER 4

CHARACTERIZATION AND SIMULTANEOUS DETERMINATION OF DECALINS IN RED YEAST RICE BY UHPLC-Q-TOF-MS AND UHPLC-QQQ-MS.....	115
--	------------

4.1 INTRODCUTION.....	115
-----------------------	-----

4.2 EXPERIMENTAL.....	117
-----------------------	-----

4.2.1 Reagents and Chemicals.....	117
-----------------------------------	-----

4.2.2 Sample Preparation.....	117
-------------------------------	-----

A
T
—
—
—
I
I
I
I

4.2.3 UHPLC-Q-TOF-MS Analysis.....	118
4.2.4 UHPLC-QQQ-MS Analysis.....	119
4.3 RESULTS AND DISCUSSION.....	120
4.3.1 Mass Fragmentation Patterns of Decalins.....	120
4.3.1.1 Fragmentation Type A.....	122
4.3.1.2 Fragmentation Type B.....	124
4.3.2 Characterization of Two New Decalins in Red Yeast Rice.....	125
4.3.3 Quantification of Decalins in Red Yeast Rce.....	128
4.3.3.1 Optimization of MS/MS Conditions.....	129
4.3.3.2 Selection of Mobile Phase.....	130
4.3.3.3 Optimization of Extraction Procedure	130
4.3.3.4 Method Validation.....	131
4.3.3.5 Analysis of Red Yeast Rice Samples.....	134
4.4 CONCLUSION.....	135

CHAPTER 5

SUMMARY AND PROSPECT OF THE RESEARCH.....	136
5.1 SUMMARY OF THE RESEARCH.....	136
5.1.1 Isolation, Structural Elucidation and Cytotoxicity Evaluation of Monacolins.....	138
5.1.2 Isolation, Structural Elucidation and Immunosuppression Evaluation of Decalins.....	139
5.1.3 Characterization and Simultaneous LC-MS Determination of Decalin Derivatives in Red Yeast Rice.....	141
5.1.4 Conclusions.....	142

5.2 PROSPECTS OF THE FUTURE STUDIES.....	145
5.2.1 Systematic Study of Structure Activity Relationship (SAR) for the Cytotoxic Effect of Dehydramonacolins.....	145
5.2.2 Examination of the Protein Profiles related to the Dehydramonacolin- induced Anti-proliferative Effect by Proteomic Analysis.....	146
5.2.3 Isolation of More Decalins from Red Yeast Rice and Discovery of More Potential Bioactivities.....	146
5.2.4 Pharmacokinetics and Metabolism Study on New Dehydromonacolins and Decalins.....	147
REFERENCES.....	149
PUBLICATIONS.....	159
PRESENTATIONS AND ABSTRACTS.....	160
CURRICULUM VITAE.....	161