UNIVERSITI PUTRA MALAYSIA

BIOTRANSFORMATION OF ZERUMBONE AND GONIOTHALAMIN, AND PHYTOCHEMICAL INVESTIGATION OF *MARCHANTIA POLYMORPHA*

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BIOTRANSFORMATION OF ZERUMBONE AND GONIOTHALAMIN, AND PHYTOCHEMICAL INVESTIGATION OF *MARCHANTIA POLYMORPHA*

By

CHIA POH WAI

Thesis Submitted to the School of Graduate Studies, University Putra Malaysia, in Fulfillment of the Requirements for the degree of Master of Science.

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Dedicated to
My Dearest Parents;
Mentor, Su-yin and Friends.

Abstract of thesis presented to the Senate of University Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

BIOTRANSFORMATION OF ZERUMBONE AND GONIOTHALAMIN BY *ASPERGILLUS NIGER* (FTCC 5003) AND *SCLEROTIUM ROLFSII* (LOCAL ISOLATED): STRUCTURAL ELUCIDATION AND BIOLOGICAL ACTIVITIES

By

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November 2007

Chairman : Associate Professor Dr. Khozirah Shaari

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Microbial transformation was carried out on three natural product compounds, namely zerumbone, goniothalamin and cardamonin, using the fungi *Aspergillus niger* and *Sclerotium rolfsii*. *Aspergillus niger* and *Sclerotium rolfsii* have successfully converted zerumbone into two products, namely 2,3-dihydrozerumbone and 6,7-epoxyzerumbone. *Aspergillus niger* and *Sclerotium rolfsii* also successfully biotransformed goniothalamin into 2,3-dihydrogoniothalamin. The trial carried out on cardamonin was unsuccessful due to the antimicrobial activity of the compound that inhibited the growth of the fungi. *Sclerotium rolfsii* is a newly discovered biotransformer able to carry out hydrogenation and epoxidation.

Two microbial toxins, namely gliotoxin and bisdethiobis(methylthio)gliotoxin were isolated in the course of biotransformation of goniothalamin using *A. niger* and *S. rolfsii* cultured on PKC (Palm Kernel Cake) media. The toxins were not produced when the

biotransformation was repeated using fungi cultured on standard glucose media. Thus, PKC was deduced to be conducive to the production of the toxins by the two fungi.

In an attempt to isolate and purify marchantin A as a candidate for biotransformation studies, several compounds which included marchantin A, apigenin, luteolin and a mixture of stigmasterol and β-sitosterol were found..

A preliminary antiinflammatory and anticholinesterase activities screening of the isolated compounds (zerumbone, 2,3-dihydrozerumbone, 6,7-epoxyzerumbone, goniothalamin, 2,3-dihydrogoniothalamin, gliotoxin, bisdethiobis(methylthio)gliotoxin, apigenin, luteolin, marchantin A and mixture of β-sitosterol and stigmasterol, were also conducted. Luteolin exhibited strong inhibition of soybean lipoxygenase with an IC_{50} value of 6.25 μg/ml while zerumbone showed moderate inhibition against soybean lipoxygenase with an IC_{50} value of 22.83 μg/ml. The other compounds were inactive towards the enzyme. In the anticholinesterase inhibitory assay, zerumbone, 6,7-epoxyzerumbone, marchantin A, goniothalamin, apigenin and luteolin, as well as a mixture of β-sitosterol and stigmasterol were found to inhibit the enzyme.

Abstrak tesis yang dikemukakan kepada senat University Putra Malaysia bagi memenuhi keperluan Ijazah Master Sains

BIOTRANSFORMASI ZERUMBON DAN GONIOTALAMIN OLEH *ASPERGILLUS NIGER* (FTCC 5003) DAN *SCLEROTIUM ROLFSII* (PENGASINGAN TEMPATAN): ELUSIDASIS STRUKTUR DAN BIOAKTIVITI

Oleh

CHIA POH WAI

November 2007

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Transformasi Mikrob telah dijalankan terhadap tiga sebatian semulajadi yang dipilih iaitu, zerumbone, goniothalamin, dan cardamonin dengan menggunakan *Aspergillus niger* dan *Sclerotium rolfsii*. *Aspergillus niger* dan *Sclerotium roflsii* berjaya mentransformasi zerumbone kepada dua hasil, iaitu 2,3-dihydrozerumbone dan 6,7-epoksizerumbone. Transformasi mikrob *Aspergillus niger* dan *Sclerotium rolfsii* juga berjaya menukarkan goniothalamin kepada 2,3-dihydrogoniothalamin. Kajian terhadap cardamonin tidak berjaya disebabkan oleh kesan antifungi yang terdapat dalam sebatian tersebut. *Sclerotium rolfsii* dikesan sebagai agen biotransformasi yang berpotensi untuk dikaji dalam transformasi mikrob.

Dua sebatian toksik mikrob, iaitu gliotoxin dan bisdethiobis(methylthio)gliotoxin telah dipencilkan daripada transformasi *Aspergillus niger* terhadap goniothalamin yang difermentasi dalam Palm Kernal Cake (PKC). Toksin mikrob ini didapati tidak

dihasilkan semula apabila kaedah transformasi mikrob diulangi dengan fungi yang dikultur dalam media asas. Oleh yang demikian, PKC dapat dicadangkan sebagai media yang kondusif untuk menghasilkan toksin mikrob tersebut dengan menggunakan fungi ini.

Dalam percubaan untuk memencilkan dan mengasingkan marchantin A sebagai calon dalam kajian biotransformasi, beberapa sebatian termasuk marchantin A, apigenin, luteolin dan campuran β-sitosterol dan stigmasterol telah ditemui.

Kajian awal aktiviti antioksidan dan antikolinesterase telah dijalankan secara teliti terhadap sebelas sebatian kimia (zerumbon, 2,3-dihydrozerumbone, 6,7-epoxyzerumbone, goniothalamin, 2,3-dihydrogoniothalamin, gliotoxin, bisdethiobis(methylthio)gliotoxin, apigenin, luteolin, marchantin A dan campuran β-sitosterol dan stigmasterol) . Luteolin telah menunjukkan activiti yang ketara terhadap kesan antioksidan dengan IC_{50} 6.25 μg/m, manakala zerumbon menunjukkan aktiviti perencatan yang sederhana terhadap enzim oksidan ini dengan IC_{50} 22.83 μg/ml. Sebatian kimia lain didapati tidak aktif terhadap aktiviti antioksidan. Dalam aktiviti perencatan antikolinesterase, zerumbon, 6,7-epoxyzerumbone, marchantin A, campuran β-sitosterol dan stigmasterol ,goniothalamin, apigenin dan luteolin didapati merencatkan enzim kolinesterase.

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I wish to express my hearty gratitude to my parents, brother and sister for their constant encouragement and sacrifice throughout this study.

Finally, I am grateful to Su-yin for her loving support and inspiration during the course of this higher study.

I certify that an Examination Committee met on _____ conduct the final examination of Chia Poh Wai on his Master of Science thesis entitled: " Biotransformation of Zerumbone and Goniothalamin by *Aspergillus niger* (FTCC 5003) and *Sclerotium rolfsii* (Local Isolated): Structural Elucidation and Biological Activities " in accordance with University Pertanian Malaysia (Higher Degree) Act 1980 and University Pertanian Malaysia (Higher Degree) Regulation 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of University Putra Malaysia and has been accepted as fulfillment in the requirements for the degree of Master Science. The members of the supervisory committee are as follows:

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DECLARATION

I hereby declared that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

_____

CHIA POH WAI

Date:

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LIST OF ABBREVIATIONS

α	Alpha
β	Beta
γ	Gama
δ	Delta or chemical shift in ppm
λ_{max}	Maximum walength in nm
μg	Microgame
μl	Microliter
br	Broad
^{13}C	Carbon-13
°C	Degree in celcius
CC	Column chromatography
$CDCl_3$	Deuterated chloroform
$CDOD_3$	Deuterated methanol
cm^{-1}	Per centimeter
d	Doublet
dd	Doublet of doublets
DCM	Dichlromethane
DEPT	Distortionless Enhancement by Polirisation Transfer
ETOAc	Ethyl Acetate
EtOH	Etanol
EIMS	Electron Impact Mass Spectroscopy
g	Grame
^{1}H	Proton

HMBC	Hetero multiplet correlation
L	Litre
M	Multiplet
mg	Miligram
m.p	Melting point
NMR	Nuclear Magnetic Resonance
s	Singlet
t	Triplet
TLC	Thin layer Chromatography
IR	Infrared
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

Currently, there are increasing interests in synthetic chemistry in the search of new lead for drug development. Synthetic chemistry has played an important role in increasing an ever-growing array of complex, optically active natural products and their analogues. It is understandable that the stereoselective elaboration of chiral centers has become a central issue, especially in pharmaceutical research and drug manufacturing.

In order to meet this challenge, there was an unprecedented development in the methodology of synthetic organic chemistry. The use of metallo-organic compounds, metal catalyzed homogeneous and heterogeneous catalysis, phase transfer catalysis, electrochemical and photochemical techniques, reagents on solid supports and other method have all contributed to the armory of stereoselective synthesis. At the same time, biotransformation via the use of enzymes and microorganisms to facilitate the reaction process has achieved significance most notably in the preparation of key chiral intermediates.

Biotransformation has emerged as a new technology in the field of natural products. It is a method of maximizing diversity from a single chemical entity by using other biological systems, in the form of whole cells or isolated enzymes, to modify the molecule. In some cases, biotransformation has become a powerful alternative to conventional synthetic chemical techniques, due to their high chemo-, region- and

stereoselectivity. More over, the ability of enzymes or microbial catalysts to perform difficult chemical reactions, such as hydroxylation of non-activated carbons, on structurally complex molecules, without the need of protection and deprotection steps of reactive functional groups, was recognized.

There are many reasons worth trying for exploiting biotransformation in natural product chemistry. Firstly, biotransformation that employs microorganisms may achieve high selective transformation of prototype compounds leading to products which are rare, or only available in very low yield by traditional chemical approaches. Secondly, interesting new analogues of biologically active compounds may be prepared without resorting to new and cumbersome total synthetic chemical methods.

Biotransformation encompasses the use of living organisms such as fungi, bacteria, microalgae, yeast and plant cell as biocatalysts. The substrate specificity of many of these microorganisms for bioconversion is often quite low, enabling a single organism to biotransform a range of related compounds with similar efficiencies. Moreover, enzymes that are produced by microorganisms are well known for practically every type of chemical reaction. There have been many examples which describe the versatility of the microbe in biotransforming chemical entities. One of the earliest and most famous is the biotransformation of progesterone (**1**) to 11 α-hydroxyprogesterone (**2**) by *Rhizopodus arrhizus* (Figure 1). The reactions are stereospecific, the ultimate in specificity being amplified by such steroid bioconversions. The end-product of this biotransformation has been widely utilized as an intermediate in the preparation of ascorbic acid (Lehman at al., 2001).

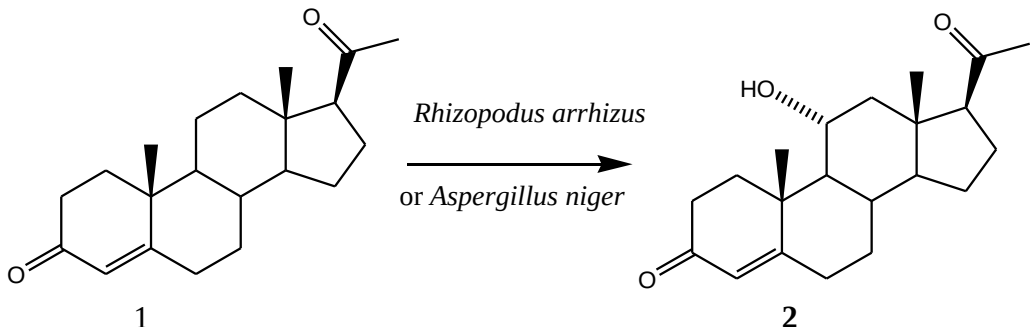

Figure 1. Biotransformation of progesterone to 11 α-hydroxyprogesterone by *Rhizopodus arrhizus* (Lehman *et al*., 2001**).**

Lately, there have been many examples of active metabolites resulting from biotransformation reaction that have been developed and marketed as drug. For example, desloratadine or Clarinex is an anti-histamine agent 10-fold more potent than its parent molecule loratadine or Claritin. An active metabolite can also serve as a modified lead compound as exemplified by ezetimibe, a cholesterol absorption inhibitor. The drug was discovered as a result of structural modification of its lead candidate SCH48461, a biotransformed product with 40-fold more potency than its parent molecule (Fura A., 2006).

In addition, biotransformation is enjoying an increasing interest, not only in academia, but also in industry. The number of synthetic steps employed in classical chemical synthesis may be reduced to a single step by biotransformation. This thereby, cuts off the occupation time of chemical reactors, which is an important factor of process economy in pharmaceutical and chemical production. The waste stream from biotransformation reactions are thus much less costly to dispose of than those of standard chemical techniques due to the fact that they are all environmental friendly by-

products. As a result, biotransformation is often considered “green chemistry” because of lower negative impact of this technology to the environment.

Malaysia, widely-recognized as one of the centers of biological diversity, is richly endowed with plants, animals and microbial genetic resources. If exploited and managed wisely, these genetic resources could provide renewable useful products for not only the present but also the future generation. This study was thus carried out with the following objectives:

1. to isolate and purify selected bioactive natural products as substrate for biotransformation,
2. to carry out microbial transformation on the selected natural products,
3. to elucidate the structures of the biotransformed products, and
4. to subject the biotransformed product to selected bioassay.