

การเปลี่ยนแปลงโครงสร้างโดยกระบวนการทางชีวภาพของกรดโคโรโนอิก

ด้วย *Psilocybe cubensis*

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต

สาขาวิชาเทคโนโลยีชีวภาพ

คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2549

ลิขสิทธิ์ของ จุฬาลงกรณ์มหาวิทยาลัย

**BIOTRANSFORMATION OF KAURENOIC ACID BY *Psilocybe cubensis***

**Miss Jaraslak Pechwang**

**A Dissertation Submitted in Partial Fulfillment of the Requirements  
for the Degree of Doctor of Philosophy Program in Biotechnology**

**Program in Biotechnology**

**Faculty of Science**

**Chulalongkorn University**

**Academic Year 2006**


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**491667**


**Thesis Title**                   BIOTRANSFORMATION OF KAURENOIC ACID BY  
*Psilocybe cubensis*  
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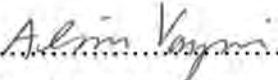
Accepted by the Faculty of Science, Chulalongkorn University in Partial  
Fulfillment of the Requirements for the Doctoral Degree

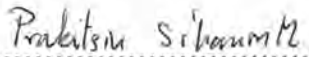
  
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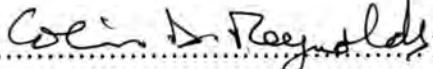
  
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จรัสลักษณ์ เพชรวัง : การเปลี่ยนแปลงโครงสร้างโดยกระบวนการทางชีวภาพของกรดโคเรโนอิกด้วย *Psilocybe cubensis*. (BIOTRANSFORMATION OF KAURENOIC ACID BY *Psilocybe cubensis*) อ. ที่ปรึกษา : รศ.ดร. อมร เพชรสม, อ.ที่ปรึกษาร่วม : ผศ.ดร. อลิสา วังโน, 150 หน้า.

การเปลี่ยนโครงสร้างทางเคมีของกรดโคเรโนอิก (*ent-kaur-16-en-19-oic acid*) ซึ่งเป็นสารออกฤทธิ์ทางชีวภาพที่สกัดได้จากเปลือกต้นเปล้าใหญ่ *Croton oblongifolius* Roxb. โดยอาศัย *Psilocybe cubensis* ให้ผลิตภัณฑ์จากปฏิกิริยาการเติมหมู่ไฮดรอกซิลในโครงสร้างหลัก 3 ชนิด คือ *ent-16 $\beta$ ,17-dihydroxy-kauran-19-oic acid* แยกได้หลังการบ่มเป็นเวลา 2 วัน และเมื่อบ่มเป็นเวลา 9 วัน พบผลิตภัณฑ์ซึ่งเป็นสารชนิดใหม่อีก 2 ชนิด คือ *ent-12 $\alpha$ ,16 $\beta$ ,17-trihydroxy-kauran-19-oic acid* และ *ent-11 $\alpha$ ,16 $\beta$ ,17-trihydroxy-kauran-19-oic acid* โครงสร้างของสารประกอบทั้งสามพิสูจน์เอกลักษณ์โดยอาศัยเทคนิคทางสเปกโตรสโคปี และวิเคราะห์โครงสร้างผลึกโมเลกุลสามมิติ การทดสอบฤทธิ์ทางชีวภาพในการยับยั้งเซลล์มะเร็ง 6 ชนิด ได้แก่ K562 (เม็ดเลือดขาว) SW620 (ลำไส้) BT474 (เต้านม) KATO-3 (กระเพาะอาหาร) HEP-G2 (ตับ) และ CHAGO (ปอด) และการยับยั้งเชื้อจุลินทรีย์ 4 ชนิด ได้แก่ *Bacillus cereus*, *Staphylococcus aureus* ATTC 25923, *Escherichia coli* ATTC 25922 and *Pseudomonas aeruginosa* ATTC 27853 พบว่าสารผลิตภัณฑ์ทุกชนิดมีฤทธิ์ยับยั้งเซลล์มะเร็งทั้ง 6 ชนิดที่ทดสอบต่ำกว่าสารตั้งต้น และไม่มีฤทธิ์ในการยับยั้งเชื้อจุลินทรีย์ทั้ง 4 ชนิดที่ความเข้มข้นที่ทดสอบ การศึกษาเอนไซม์ที่เกี่ยวข้องในการเติมหมู่ไฮดรอกซิลในโครงสร้างของกรดโคเรโนอิกพบว่า เอนไซม์ถูกสร้างเมื่อราถูกกระตุ้นด้วยกรดโคเรโนอิก โดยตำแหน่งเอนไซม์พบในส่วน microsomal และต้องการ NADPH และ FAD เป็น cofactor ในการเร่งปฏิกิริยา การวิเคราะห์ชนิดของโปรตีนด้วยเทคนิค MALDI/Tof MS และ peptide mass mapping พบว่าโปรตีนขนาด 45 kDa ซึ่งแยกได้จากเทคนิค SDS-PAGE มีส่วนของเปปไทด์สอดคล้องกับลำดับเปปไทด์ของ cytochrom P450 monooxygenase ที่พบใน *Aspergillus fumigatus* Af293 โดยมีลำดับที่สอดคล้องกัน 12 %

สาขาวิชา.....เทคโนโลยีชีวภาพ.....ลายมือชื่อนิสิต.....จรัสลักษณ์ เพชรวัง  
ปีการศึกษา.....2549.....ลายมือชื่ออาจารย์ที่ปรึกษา.....อมร เพชรสม  
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## 4473855823 : MAJOR BIOTECHNOLOGY

KEY WORD: BIOTRANSFORMATION / *Psilocybe cubensis* / KAURENOIC ACID

JARASLAK PECHWANG : BIOTRANSFORMATION OF KAURENOIC ACID BY *Psilocybe cubensis*. THESIS ADVISOR : ASSOC. PROF. AMORN PETSOM, Ph.D, THESIS COADVISOR : ASST. PROF. ALISA VANGNAI, Ph.D., 150 pp.

Biotransformation of *ent*-kaur-16-en-19-oic acid (**1**), the bioactive kaurene diterpenoid isolated from the stem bark of *Croton oblongifolius* Roxb., was carried out using *Psilocybe cubensis*. The incubation resulting in three hydroxylated products; *ent*-16 $\beta$ ,17-dihydroxy-kauran-19-oic acid (**2**) was isolated after 2 days of incubation and the two novel metabolites; *ent*-12 $\alpha$ ,16 $\beta$ ,17-trihydroxy-kauran-19-oic acid (**3**) and *ent*-11 $\alpha$ ,16 $\beta$ ,17-trihydroxy-kauran-19-oic acid (**4**) were obtained after incubation for 9 days. The metabolites were identified by spectroscopic methods and X-ray crystallography. The biological activities of each compound were evaluated by cytotoxicity test against 6 tumor cell lines; K562 (human promyelocytic leukemia cells), HEP-G2 (hepatoma), SW620 (colon), Chago (lung), KATO-3 (gastric) and BT474 (breast) and anti-bacteria activity against 4 bacteria; *Bacillus cereus*, *Staphylococcus aureus* ATTC 25923, *Escherichia coli* ATTC 25922 and *Pseudomonas aeruginosa* ATTC 27853. The results revealed that all products exerted lower levels of biological activities than their parent compound in cytotoxicity testing towards 6 cell lines and were inactive for anti-bacteria activity. The enzyme responsible for compound **2** production was inducible in 2-day grown culture supplemented with *ent*-kaurenoic acid. The enzyme has been identified as an induced oxygenase requiring NADPH and FAD as cofactors and located in the microsomal fraction. The 45 kDa protein from SDS-PAGE was selected for further analysis by MALDI/Tof MS and peptide mass mapping. The result showed the correlation of the peptide fragment with cyt P450 monooxygenase from *Aspergillus fumigatus* Af293 at sequence coverage of 12 %.

Field of study.....Biotechnology.....Student's signature.....Jaraslak Pechwang  
 Academic year.....2006.....Advisor's signature.....  
 Co-advisor's signature.....Alisa Vangnai.....

## ACKNOWLEDGEMENTS

I wish to express my deepest appreciation to my advisor, Associate Professor Amorn Petsom for his encouraging guidance, supervision and beneficial suggestions throughout the course of this research. My gratitude is also extended to my co-advisor Assistant Professor Alisa Vangnai for her instruction and guidance. Special thanks are extended to Professor Sophon Roengsumran for helpful discussion and suggestions throughout my study and serving as chairman of my thesis committee.

My appreciation is also expressed to Associate Professor Prakitsin Sihanonth, Assistant Professor Surachai Pornpakakul, Dr. Jitra Piapukiew, and Professor Colin D. Renolds for their kindness and helpful suggestion for the complements of this thesis and serving as dissertation committee. I am very grateful to Professor Anthony Whalley and Dr. Edward Lock, Faculty of Science, Liverpool John Moores University, UK. for their kindness, helpful and invaluable suggestions. I would like to express my sincere thanks to Assistant Professor Polkrit Sangvanich and Dr. Ek Sangvichien for their help and advice. Thanks also to Dr. Siriporn Chuchawankul, Faculty of Allied Health Science, Chulalongkorn University for cytotoxicity testing.

My sincere gratitude is also extended to the Royal Golden Jubilee Ph.D. Scholarship for the financial support and the Program of Biotechnology and the Graduate School, Chulalongkorn University for partial financial support. Thanks to the Department of Chemistry, Faculty of Science for laboratory facilities and the Department of Transfusion medicine, Faculty of Allied Health Science, Chulalongkorn University for the facility for anti-microbial evaluation.

Sincere thanks are extended to all staff members and friends in the Program of Biotechnology and the Department of Chemistry, Faculty of Science, Chulalongkorn University especially in the Research Centre of Bioorganic Chemistry including all staff and friends in the School of Biomolecular Sciences, Faculty of Science, Liverpool John Moores University, UK. for all their kindness, help and friendship.

Finally, I wish to express my deepest gratitude to my parents for all things that they have endured and sacrificed for my success.

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## ABBREVIATIONS

$[\alpha]_D^{20}$	Specific rotation at 20 °C and Sodium D line (589 nm)
brs	Broad singlet (for NMR spectral data)
°C	Degree Celsius
$^{13}\text{C-NMR}$	Carbon -13 nuclear magnetic resonance
$\text{CD}_3\text{OD}$	Deuterated methanol
cm	Centimeter
COSY	$^1\text{H-}^1\text{H}$ correlation spectroscopy
$\delta$	Chemical shift
d	Doublet (for NMR spectral data)
dd	Doublet of doublet (for NMR spectral data)
DMSO	Dimethyl sulfoxide
dt	Doublet of triplet (for NMR spectral data)
DW	Distilled water
EtOAc	Ethyl acetate
g	Gravitational acceleration
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Coherence
$^1\text{H-NMR}$	Proton Nuclear Magnetic resonance
IR	Infrared spectroscopy
kDa	kilo Dalton
l	Liter
$\mu$	Micro liter
$[\text{M}+\text{H}]^+$	Protonated molecular ion
m	Multiple (for NMR spectral data)
mA	Milliamp
mg	Milligram
$\mu\text{g}$	Microgram
MHz	Megahertz
ml	Milliliter
M	Molar
mM	Millimolar

M	Molar
mM	Millimolar
mmol	millimole
MW	Molecular weight
MeOH	Methanol
$\nu_{\max}$	Wave number at maximum absorption
$(\text{NH}_4)_2\text{SO}_4$	Ammonium sulfate
NMR	Nuclear magnetic resonance
PDA	Potato dextrose agar
s	singlet (for NMR spectral data)
SDS	Sodium dodecyl sulfate
t	Triplet (for NMR spectral data)
TLC	Thin layer Chromatography
wet wt	wet weight
UV	Ultraviolet