

การสกัดให้บริสุทธิ์และการศึกษาคุณสมบัติของเอนไซม์ที่ทำหน้าที่เชื่อมระหว่าง
โมเลกุลของโตปามีนและเซคโคโลกานินจากใบปอู้

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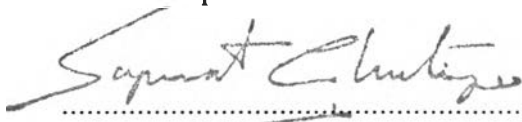
**PURIFICATION AND CHARACTERIZATION OF
DOPAMINE-SECOLOGANIN CONDENSING ENZYME
FROM *ALANGIUM SALVIIFOLIUM* WANG
SSP. *HEXAPETALUM* WANG LEAVES**

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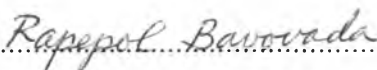
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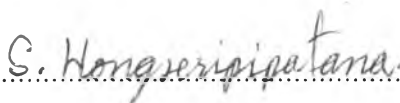

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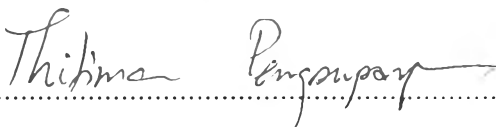
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เอนไซม์ที่ทำหน้าที่เร่งปฏิกิริยาการเชื่อมระหว่างโมเลกุลของโดปามีน (Dopamine) และเซคโคโลแกนิน (Secologanin) แล้วได้ดีอะเซทิลไอเพคโคไซด์ (*R*-Deacetylpecoside) ซึ่งมีโครงสร้างแบบอาร์ จากใบปอู้ ได้ถูกสกัดแยกให้บริสุทธิ์โดยการตกตะกอนด้วยแอมโมเนียมซัลเฟต (Ammonium sulfate), อุลตราฟิลเตรชัน (Ultrafiltration) และผ่าน 3 ชั้นตอนของคอลัมน์โครมาโตกราฟี (Column chromatography) สามารถตรวจสอบความบริสุทธิ์โดยอิเล็กโตรโฟรีซิส (Electrophoresis) พบว่าเอนไซม์ที่แยกได้ประกอบด้วยสายโพลีเพปไทด์สายเดี่ยว มีน้ำหนักโมเลกุล 30,000 Da มีสภาวะที่เหมาะสมต่อการทำงานที่ pH 7.5 และอุณหภูมิ 37°C ผลการศึกษาทางจลนศาสตร์ของเอนไซม์พบว่าเอนไซม์ มีค่า k_m 0.69 mM สำหรับโดปามีน และ 0.92 mM สำหรับเซคโคโลแกนิน และ V_{max} 7.09 $\mu\text{kat}/\text{mg}$ protein สำหรับโดปามีนและ 8.33 $\mu\text{kat}/\text{mg}$ protein สำหรับเซคโคโลแกนิน เอนไซม์เร่งปฏิกิริยาจำเพาะเจาะจงต่อโดปามีนสูงเนื่องจากไม่สามารถเร่งปฏิกิริยาที่มีไทรามิน (Tyramine) และทริปตามีน (Tryptamine) รวมทั้งไม่ถูกยับยั้งการเร่งปฏิกิริยาเมื่ออยู่ในสภาวะที่สารตั้งต้นมีปริมาณมาก การทำงานของเอนไซม์จะถูกยับยั้งการเร่งปฏิกิริยาโดยอะแลงจิมารคคิน (Alangimarckine) และดีไฮโดรอะแลงจิมารคคิน (Dehydroalangimarckine) โดยมี IC_{50} ประมาณ 10 μM ผลิตผลของการเชื่อมระหว่างโมเลกุลของโดปามีนและเซคโคโลแกนินจะอยู่ในรูปของดีเมทิลอะแลงจิด (Demethylalangiside) ซึ่งเปลี่ยนแปลงโครงสร้างมาจากดีอะเซทิลไอเพคโคไซด์ (Deacetylpecoside) ระหว่างขบวนการสกัด จากผลการวิจัยที่ได้ จึงตั้งชื่อเอนไซม์ที่ใช้ในการเชื่อมโมเลกุลของโดปามีนและเซคโคโลแกนินนี้ว่า "Deacetylpecoside synthase" เชื่อว่าจะเป็นเอนไซม์ตัวแรกของวิธีชีวสังเคราะห์ของแอลคาลอยด์ในกลุ่มเตตราไฮโดรไอโซควิโนลีน โมโนเทอร์ปีน กลูโคไซด์ (Tetrahydroisoquinoline monoterpene glucosides) ทั้งหลายซึ่งมีโครงสร้างแบบอาร์เช่นเดียวกัน

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สาขาวิชา เลขชี้แจง.....
ปีการศึกษา 2541.....

ลายมือชื่อนิสิต
ลายมือชื่ออาจารย์ที่ปรึกษา
ลายมือชื่ออาจารย์ที่ปรึกษาพร้อม

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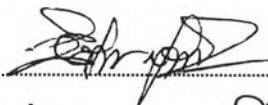
KEY WORD: ALANGIACEAE / *ALANGIUM SALVIIFOLIUM* WANG / DEACETYLIPECOSIDE / DEACETYLIPECOSIDE SYNTHASE / ISOQUINOLINE MONOTERPENE ALKALOID BIOSYNTHESIS/ EMETINE ALKALOID / CHARACTERIZATION / PURIFICATION NITIMA SUTTIPANTA : PURIFICATION AND CHARACTERIZATION OF DOPAMINE-SECOLOGANIN CONDENSING ENZYME FROM *ALANGIUM SALVIIFOLIUM* WANG SSP. *HEXAPETALUM* WANG LEAVES THESIS ADVISOR : ASSOC. PROF. WANCHAI DE-EKNAMKUL, Ph.D. 103 pp. ISBN 974-639-698-6


Dopamine-secologanin condensing enzyme, the enzyme catalyzing the condensation of dopamine and secologanin to form the (*R*)-epimer of deacetylipecoside, has been purified from the leaves of *Alangium salviifolium* Wang. The enzyme was purified to apparent electrophoretic homogeneity by ammonium sulfate precipitation, ultrafiltration and three subsequent column chromatography steps. The isolated enzyme is a single polypeptide with M_r 30,000 and has a pH optimum at 7.5 and a temperature optimum at 37°C. The apparent K_m value for dopamine and secologanin are 0.69 mM and 0.92 mM, respectively. The V_{max} for dopamine and secologanin are 7.09 and 8.33 pkat/mg protein, respectively. The enzyme has high substrate specificity to dopamine; neither tyramine nor tryptamine are utilized by the enzyme. No substrate inhibition was observed. The enzyme activity is inhibited by alangimarckine and dehydroalangimarckine with similar IC_{50} value approximately of 10 mM. The enzymatic product was confirmed to be demethylalangiside which is the spontaneous lactamization product of (*R*)-deacetylipecoside. From these results, the dopamine-secologanin condensing enzyme was named "deacetylipecoside synthase" which presumably catalyzes the provision of (*R*)-deacetylipecoside for the formation of tetrahydroisoquinoline monoterpene glucosides that possess also (*R*)-configuration at the same chiral center.

ภาควิชา..... เกษัชเวช.....

สาขาวิชา..... เกษัชเวช.....

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ลายมือชื่ออาจารย์ที่ปรึกษา..... 

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ABBREVIATIONS

AA	=	acrylamide
APS	=	ammonium persulfate
AUFS	=	absorbance full scale
Bis	=	N,N',methylene bisacrylamide
BSA	=	bovine serum albumin
cDNA	=	cloning DNA
cm	=	centimeter
cpm	=	counts per minute
DEAE	=	diethylaminoethyl
dpm	=	disintegrations per minute
EDTA	=	ethylenediamine tetraacetic acid
eg.	=	for example
etc.	=	et cetera
Fig	=	Figure
FPLC	=	fast protein liquid chromatography
fr.wt	=	fresh weight
g	=	gram
HPLC	=	high performance liquid chromatography
hr	=	hour
M+	=	molecular ion
m/z	=	mass to charge ratio
mA	=	miliampere
min	=	minute
ml	=	mililiter
M_r	=	molecular mass relative to 1/12 of the atomic mass ^{12}C
nm	=	nanometer(s)
no.	=	number
opt	=	optimum

pI	=	isoelectric point
pH	=	hydrogen ion concentration
pkat	=	pico katal
pmol	=	picomole(s)
R _f	=	distance spot moved/ distance solvent moved (TLC)
rpm	=	revolutions per minute
SDS	=	sodium dodecyl sulfate
SDS-PAGE	=	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
sp.act.	=	specific activity
TEMED	=	N,N,N',N'-tetramethylethylenediamine
TLC	=	thin layer chromatography
UV	=	ultraviolet light
V _e	=	elution volume
V _o	=	void volume
³ H	=	tritium
β	=	beta
°C	=	degree celsius
g	=	centrifugal force (relative to gravity)
λ _{max}	=	wavelength at maxima absorption
μCi	=	microCurie
μmol	=	micromole
N ₂	=	Nitrogen atmosphere
K _m	=	Michaelis constant = substrate concentration at which the rate of enzyme-catalysed reaction is half maximum rate
Da	=	dalton, unit of molecular mass (1/12 of C=1)
kD	=	kilodalton, (x 10 ³ Da)
V _{max}	=	maximum velocity of enzyme
μM	=	micro molar(s)
M	=	molar(s)
μg	=	microgram(s)