

องค์ประกอบทางเคมีของแก่นไม้แดง

นางสาวนิจพร วชิรัตน์พงษ์เมธิ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต

สาขาวิชาเภสัชเวช ภาควิชาเภสัชเวช

คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2550

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

CHEMICAL CONSTITUENTS OF THE HEARTWOOD OF
XYLIA XYLOCARPA VAR. *KERRII*

Miss Nidchaporn Wachirattanapongmetee

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Pharmacognosy

Department of Pharmacognosy

Faculty of Pharmaceutical Sciences

Chulalongkorn University

Academic Year 2007

Copyright of Chulalongkorn University

501872

นิพนธ์ วชิรัตน์พงษ์เมธี : องค์ประกอบทางเคมีของแก่นไม้แดง (CHEMICAL
 CONSTITUENTS OF THE HEARTWOOD OF *XYLIA XYLOCARPA* VAR.
KERRII) อาจารย์ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.ดร.ชัยโย ชัยชาญทิพพุทธ, 117 หน้า.

พบสารใหม่ที่ยังไม่เคยมีรายงานมาก่อน 2 ชนิด คือ 8(9),15-isopimaradiene-3-one, 14 β -ol และ sandaracopimaradiene-2 α ,3 β -diol จากการศึกษาองค์ประกอบทางเคมีของแก่นไม้แดง (*Xylocarpa xylocarpa* var. *kerrii*) จากจังหวัดเลยและแม่ฮ่องสอน นอกจากนี้สามารถสกัดแยกสารได้ส่วนผสมของ β -sitosterol และ stigmasterol และสารบริสุทธิ์กลุ่มไพมาเรนไดเทอร์ปีนอีก 4 ชนิด ได้แก่ sandaracopimaradiene-3 β ,18-diol, sandaracopimaradiene-3-one, sandaracopimaradiene-3 β -ol, sandaracopimaric acid การพิสูจน์เอกลักษณ์และสูตรโครงสร้างทางเคมีของสาร โดยการวิเคราะห์ข้อมูลทางสเปกโตรสโคปีจาก UV, IR, MS, 1D-NMR, 2D-NMR ร่วมกับการเปรียบเทียบข้อมูลที่ได้กับสารที่มีการรายงานมาก่อน เมื่อนำองค์ประกอบทางเคมีที่แยกได้มาทดสอบการต้านเซลล์มะเร็ง 5 ชนิดในหลอดทดลอง คือ มะเร็งกระเพาะอาหาร (KATO-3), ลำไส้ (SW620), เต้านม (BT474), ตับ (HEP-G2) และ ปอด (CHAGO) พบว่า สารกลุ่มไพมาเรนไดเทอร์ปีนคือ sandaracopimaradiene-3 β ,18-diol, sandaracopimaradiene-3-one, sandaracopimaradiene-3 β -ol, sandaracopimaric acid และ 8(9),15-isopimaradiene-3-one,14 β -ol มีฤทธิ์ต้านเซลล์มะเร็งได้แรงและปานกลาง แต่สาร sandaracopimaradiene-2 α ,3 β -diol ไม่มีฤทธิ์ในการต้านเซลล์มะเร็งทั้งหมด

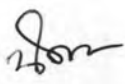
ภาควิชาเภสัชเวท.....

สาขาวิชา.....เภสัชเวท.....

ปีการศึกษา2550.....

ลายมือชื่อนิสิต

ลายมือชื่ออาจารย์ที่ปรึกษาวิทยานิพนธ์หลัก


 ชัยโย ชัยชาญทิพพุทธ

4976573833 : PHARMACOGNOSY

KEY WORD: *XYLIA XYLOCARPA* / DITERPENE / PIMARANE /

SANDARACOPIMARADIENE

MISS NIDCHAPORN WACHIRATTANAPONGMETEE : CHEMICAL
CONSTITUENTS OF THE HEARTWOOD OF *XYLIA XYLOCARPA* VAR.
KERRII. THESIS PRINCIPAL ADVISOR : ASSOC. PROF. CHAIYO
CHAICANTIPYUTH, Ph. D., 117 pp.

Two new pimarane-type diterpenoids are 8(9),15-isopimaradiene-3-one,14 β -ol and sandaracopimaradiene-2 α ,3 β -diol in the course of the investigation for chemical constituents of dried heartwood of *Xylia xylocarpa* var. *kerrii* from Loei and Mae Hong Son province. Moreover, there were a mixture of β -sitosterol and stigmasterol and 4 pimarane-type diterpenoid compounds such as sandaracopimaradiene-3 β ,18-diol, sandaracopimaradiene-3-one, sandaracopimaradiene-3 β -ol, sandaracopimaric acid. The structure of these compounds were established by analysis of their spectroscopic data (UV, IR, MS, 1D-NMR, 2D-NMR analysis) as well as comparison with previously reported values. Each compound was tested *in vitro* cytotoxicity against 5 human cancer cell lines; KATO-3 (human gastric carcinoma), SW620 (human colon adenocarcinoma), BT474 (human breast ductal carcinoma), HEP-G2 (human liver hepatoblastoma), CHAGO (human undifferentiated lung carcinoma). The pimarane-type diterpenoids such as sandaracopimaradiene-3 β ,18-diol, sandaracopimaradiene-3-one, sandaracopimaradiene-3 β -ol, sandaracopimaric acid and 8(9),15-isopimaradiene-3-one,14 β -ol showed strong and moderate cytotoxic activity against 5 human cancer cell lines but sandaracopimaradiene-2 α ,3 β -diol was inactivated all tested cancer cell lines.


DepartmentPharmacognosy.....

Student's signature

Field of study ...Pharmacognosy.....

Advisor's signature

Academic year2007...


Chaiyo Chaichantipyuth

ACKNOWLEDGEMENTS

I wish to express my grateful appreciation to my thesis advisor. Associate Professor Chaiyo Chaichantipyuth, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for his guidance, suggestion, encouragement, and kindness throughout the research studies.

I would like to acknowledge my sincere thanks to Mrs. Songchan Puthong, The Institute of Biotechnology and Genetic Engineering, for her concern, kindly assistance about cytotoxicity test.

I would like to acknowledge my sincere thanks to Dr. Rapepol Bavovada Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences. Chulalongkorn University, for his concern, kindly assistance and valuable advice.

I would like to thank the members of my thesis examination committee, for their valuable comments, useful suggestion and for serving on my examination.

I would also like to thank, with great appreciation, the Graduate school of Chulalongkorn University for granting partial financial support to conduct this investigation

I wish to thank my family, teachers, co-worker at Burapha University, Jiranuch Mingmuang and all my friends in the Department of Pharmacognosy for their contributions, hospitalities and unforgettable friendships during my study.

CONTENTS

	page
ABSTRACT (Thai).....	iv
ABSTRACT (English).....	v
ACKNOWLEDEMENTS.....	vi
CONTENTS.....	vii
LIST OF TABLES.....	x
LIST OF FIGURES.....	xii
LIST OF SCHEMES.....	xv
ABBREVIATIONS.....	xvi

CHAPTER

I INTRODUCTION.....	1
II LITERATURE REVIEW.....	2
- THE GENUS <i>XYLIA</i>	2
- Taxa and Description.....	2
- The Chemistry of the genus <i>Xylia</i>	4
- Biological activities of genus <i>Xylia</i>	6
- THE PIMARANES.....	7
- Biological properties of Pimaranes.....	13
- Biosynthesis of Pimaranes.....	17
- CYTOTOXICITY.....	18
III EXPERIMENTAL.....	19
1. Source of Plant Materials.....	19
2. General Techniques.....	19
2.1 Analytical Tin Layer Chromatography (TLC).....	19
2.2 Column Chromatography.....	19
2.2.1 Conventional Column Chromatography.....	19
2.2.2 Flash Column Chromatography.....	20

	page
2.3 Spectroscopic techniques.....	20
2.3.1 Ultraviolet (UV) Absorption Spectra.....	20
2.3.2 Infrared (IR) Absorption Spectra.....	20
2.3.3 Mass Spectra (MS).....	20
2.3.4 Proton and Carbon-13 NMR Spectra	21
2.4 Physical properties.....	21
2.4.1 Melting Points.....	21
2.4.2 Optical Rotations.....	21
2.5 Solvents.....	21
3. Extraction and Isolation.....	22
Part I : Heartwood of <i>X. xylocarpa</i> var. <i>kerrii</i> from Loei province	
1. The Extraction.....	22
2. The Separation and Isolation.....	23
Part II : Heartwood of <i>X. xylocarpa</i> var. <i>kerrii</i> from Mae Hong Son province	
1. The Extraction.....	30
2. The Separation and Isolation.....	30
4.. Physical and Spectral Data of the isolated compounds.....	33
1. Compound A-1	33
2. Compound A-2	33
3. Compound A-3.....	34
4. Compound A-4.....	34
5. Compound B-1	35
6. Compound B-2.....	35
7. Compound B-3.....	36
8. Compound B-4.....	36
9. Compound B-5.....	37
5. Cytotoxicity test.....	38

	page
IV RESULT AND DISCUSSION.....	39
1. Structure And Determination of the isolated compounds	
Part I : Heartwood of <i>X. xylocarpa</i> var. <i>kerrii</i> from	
Loei province	39
Part II : Heartwood of <i>X. xylocarpa</i> var. <i>kerrii</i> from	
Mae Hong Son province.....	51
2. Cytotoxicity.....	60
V CONCLUSION.....	63
REFERENCES.....	64
APPENDICES.....	69
VITA.....	117

LIST OF TABLES

TABLE	page
1. Combination of fractions from crude hexane extract by conventional column chromatography	23
2. Combination of fractions from fraction H-I (13.2621 g) by column chromatography	24
3. Combination of fractions from fraction HI-1 (9.4142 g) by flash column chromatography	24
4. Combination of fractions from fraction I-3 (1.7930 g) by flash column chromatography	25
5. Combination of fractions from fraction HI-2 (1.1918 g) by flash column chromatography	26
6. Combination of fractions from fraction crude ethyl acetate extract (12 g) by conventional column chromatography.....	27
7. Yield of the hexane extract and ethyl acetate extract of <i>X. xylocarpa</i> var. <i>kerrii</i> from Loei province	29
8. Combination of fractions from crude hexane extract (6.7 g) by conventional column chromatography.....	30
9. Yield of the hexane extract and ethyl acetate extract of <i>X. xylocarpa</i> var. <i>kerrii</i> from Mae Hong Son province.....	32
10. The IR absorption band assignment of compound A-1.....	39
11. Comparison of the ^1H , ^{13}C NMR chemical shift assignments of compound A-1 and sandaracopimaradiene-3 β ,18-diol	41
12. The IR absorption band assignment of compound A-2.....	42
13. Comparison of the ^{13}C NMR chemical shift assignments of compound A-2 and sandaracopimaradiene-3-one.....	43
14. The IR absorption band assignment of compound A-3.....	45
15. Comparison of the ^{13}C NMR chemical shift assignments of compound A-3 and sandaracopimaradiene-3 β -ol.....	46
16. The IR absorption band assignment of compound A-4.....	47

TABLE		page
17	The ^1H NMR, ^{13}C NMR, ^1H - ^1H COSY, HMBC spectral data of compound A-4.....	49
18	Comparison of the ^{13}C NMR chemical shift assignments of compound B-2 and β -sitosterol and stigmasterol	52
19	The IR absorption band assignment of compound B-3.....	53
20	The ^{13}C NMR chemical shift assignments of compound B-3 and sandaracopimaric acid.....	55
21	The IR absorption band assignment of compound B-4.....	56
22	The ^1H NMR, ^{13}C NMR, ^1H - ^1H COSY, HMBC spectral data of compound B-4.....	58
23	Cytotoxic activity of compounds from <i>X. xylocarpa</i> var. <i>kerrii</i>	60
24	IC_{50} of active compounds from <i>X. xylocarpa</i> var. <i>kerrii</i>	61

LIST OF FIGURES

FIGURE	page
1	<i>X. xylocarpa</i> (Roxb.) Taub. var. <i>kerrii</i> (Craib & Hutch.) I.C.Nielsen.....70
2	Leaves of <i>X. xylocarpa</i> Taub. from Loei province 71
3	Leaves and heartwood of <i>X. xylocarpa</i> Taub. from Mae Hong Son province72
4	The UV spectrum of compound A-1 in MeOH 73
5	The mass spectrum of compound A-1 73
6	The IR spectrum of compound A-1 (KBr disc) 74
7	The 300 MHz ¹ H-NMR spectrum of compound A-1 (CDCl ₃)..... 75
8	The 75 MHz ¹³ C-NMR spectrum of compound A-1 (CDCl ₃)..... 76
9	The UV spectrum of compound A-2 in MeOH 77
10	The mass spectrum of compound A-2 77
11	The IR spectrum of compound A-2 (KBr disc) 78
12	The 300 MHz ¹ H-NMR spectrum of compound A-2 (in CDCl ₃)....79
13	The 75 MHz ¹³ C-NMR spectrum of compound A-2 (in CDCl ₃)....80
14	The UV spectrum of compound A-3 in MeOH..... 81
15	The mass spectrum of compound A-3 81
16	The IR spectrum of compound A-3 (KBr disc)..... 82
17	The 300 MHz ¹ H-NMR spectrum of compound A-3 (in CDCl ₃)....83
18	The 75 MHz ¹³ C-NMR spectrum of compound A-3 (in CDCl ₃)....84
19	The UV spectrum of compound A-4 in MeOH..... 85
20	The mass spectrum of compound A-4 85
21	The IR spectrum of compound A-4 (KBr disc) 86
22	The 500 MHz ¹ H-NMR spectrum of compound A-4 (in CDCl ₃)....87
23	The expanded 500 MHz ¹ H-NMR spectrum of compound A-4 (in CDCl ₃) (δ _H 1.0-2.1 ppm, δ _H 2.0-5.8 ppm).....88
24	The 125 MHz ¹³ C-NMR spectrum of compound A-4 (CDCl ₃)..... 89
25	The DEPT-135 spectrum of compound A-4 (in CDCl ₃)..... 90

FIGURE	page
26 The 75 MHz ^1H - ^1H COSY spectrum of compound A-4 (in CDCl_3).....	91
27 The 500 MHz HSQC spectrum of compound A-4 (in CDCl_3).....	92
28 The expanded 500 MHz HSQC spectrum of compound A-4 (in CDCl_3) (δ_{H} 0.8-2.1 ppm, δ_{C} 18-35 ppm).....	93
29 The 500 MHz HMBC spectrum of compound A-4 (in CDCl_3).....	94
30 The expanded 500 MHz HMBC spectrum of compound A-4 (in CDCl_3) (δ_{H} 0.8-7ppm, δ_{C} 20-75 ppm and δ_{H} 3.0-6.0 ppm, δ_{C} 20-42 ppm).....	95
31 The expanded 500 MHz HMBC spectrum of compound A-4 (in CDCl_3) (δ_{H} 1.0-2.8ppm, δ_{C} 110-220 ppm and δ_{H} 3.0-6.0 ppm, δ_{C} 110-220 ppm).....	96
32 The 300 MHz ^1H -NMR spectrum of compound B-1 (in CDCl_3)....	97
33 The 75 MHz ^1H -NMR spectrum of compound B-1 (in CDCl_3).....	98
34 The 300 MHz ^1H -NMR spectrum of compound B-2 (in CDCl_3)....	99
35 The 75 MHz ^{13}C -NMR spectrum of compound B-2 (in CDCl_3).....	100
36 The UV spectrum of compound B-3 in MeOH.....	101
37 The mass spectrum of compound B-3	101
38 The IR spectrum of compound B-3 (KBr disc)	102
39 The 300 MHz ^1H -NMR spectrum of compound B-3 (in CDCl_3)...	103
40 The 75 MHz ^{13}C -NMR spectrum of compound B-3 (in CDCl_3)....	104
41 The UV spectrum of compound B-4 in MeOH.....	105
42 The mass spectrum of compound B-4.....	105
43 The IR spectrum of compound B-4 (KBr disc).....	106
44 The 500 MHz ^1H -NMR spectrum of compound B-4 (in CDCl_3)....	107
45 The expanded 500 MHz ^1H -NMR spectrum of compound B-4 (in CDCl_3) (δ_{H} 1.0-3.7 ppm).....	108
46 The 125 MHz ^{13}C -NMR spectrum of compound B-4 (in CDCl_3)...	109
47 The DEPT-90 spectrum of compound B-4 (in CDCl_3).....	110

FIGURE		page
48	The DEPT-135 spectrum of compound B-4 (in CDCl ₃).....	110
49	The 75 MHz ¹ H- ¹ H COSY spectrum of compound B-4 (in CDCl ₃).....	111
50	The 500 MHz HSQC spectrum of compound B-4 (in CDCl ₃).....	112
51	The expanded 500 MHz HSQC spectrum of compound B-4 (in CDCl ₃) (δ _H 1.0-4.0 ppm, δ _C 35-70, ppm and δ _H 2.8-6.0 ppm, δ _C 80-150 ppm).....	113
52	The 500 MHz HMBC spectrum of compound B-4 (in CDCl ₃).....	114
53	The expanded 500 MHz HMBC spectrum of compound B-4 (in CDCl ₃) (δ _H 1.1-2.4 ppm, δ _C 14-30, ppm and δ _H 1.1-2.4 ppm, δ _C 33-58 ppm).....	115
54	The expanded 500 MHz HMBC spectrum of compound B-4 (in CDCl ₃) (δ _H 3.0-6.0 ppm, δ _C 14-55 ppm and δ _H 0.8-2.3 ppm, δ _C 63-95 ppm).....	116

LIST OF SCHEMES

SCHEME		page
1	Biosynthesis of Pimaranes.....	17
2	MTT reduction.....	18
3	Extraction of the heartwood of <i>X. xylocarpa</i> var. <i>kerrii</i>	22
4	Isolation from the hexane extract of <i>X. xylocarpa</i> var. <i>kerrii</i> from Loei province.....	28
5	Isolation from the ethyl acetate extract of <i>X. xylocarpa</i> var. <i>kerrii</i> from Loei province.....	29
6	Isolation from the hexane extract of <i>X. xylocarpa</i> var. <i>kerrii</i> from Mae Hong Son province.....	32

ABBREVIATIONS

br d	= Broad doublet (for NMR spectra)
br s	= Broad singlet (for NMR spectra)
°C	= Degree celsius
CDCl ₃	= Deuterated chloroform
¹³ CNMR	= Carbon-13 Nuclear Magnetic Resonance
cm	= Centimeter
COSY	= Correlated spectroscopy
1-D	= One dimensional (for NMR spectra)
2-D	= Two dimensional (for NMR spectra)
d	= Doublet (for NMR spectra)
dd	= Doublets of doublet (for NMR spectra)
ddd	= Doublets of doublets of doublet (for NMR spectra)
dt	= Doublets of triplet (for NMR spectra)
δ	= Chemical shift
DEPT	= Distortionless Enhancement by Polarization Transfer
ES TOFMS	= Electrospray time of flight mass spectrum
FT-IR	= Fourier Transform Infrared
g	= Gram
¹ H-NMR	= Proton Nuclear Magnetic Resonance
HMBC	= ¹ H-detected multiple bond heteronuclear multiple bond coherent
HSQC	= ¹ H-detected heteronuclear single-quantum coherent
Hz	= Hertz
IR	= Infrared
IC ₅₀	= Inhibitory Concentration 50%
<i>J</i>	= Coupling constant
KBr	= Potassium bromide
Kg	= Kilogram
λ _{max}	= Wavelength at maxima absorption

M^+	= Molecular ion
m	= Multiplet (for NMR spectra)
MeOH	= Methanol
mg	= Milligram
MHz	= Mega Hertz
ml	= Millilitre
m.p.	= Melting point
μg	= Microgram
m/z	= Mass to charge ratio
MS	= Mass spectrum
nm	= Nanometer
ppm	= Part per million
s	= Singlet (for NMR spectra)
spp.	= Species
t	= Triplet (for NMR spectra)
TLC	= Tin Layer Chromatography
UV-VIS	= Ultraviolet and Visible spectrophotometer
ν_{max}	= Wave number at maximum adsorption
ϵ	= Molar absorptivity
$[\alpha]^{25}\text{D}$	= Specific rotation at 25°C and Sodium D line (589 nm)