

ความเป็นพิษต่อเซลล์มะเร็งของส่วนประกอบทางเคมีจากเปลือกต้นเปลือกใหญ่

Croton oblongifolius Roxb. จาก อำเภอเมือง จังหวัดประจำวันศรีขันธ์

นางสาว นุญจิรา บุญทา



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาเคมี ภาควิชาเคมี

คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2543

ISBN 974-347-013-1

ลิขสิทธิ์ของ จุฬาลงกรณ์มหาวิทยาลัย

CYTOTOXICITY OF CHEMICAL CONSTITUENTS OF THE STEM BARKS OF
Croton oblongifolius Roxb. FROM MUANG, PRACHUAP KHIRI KHAN PROVINCE

Miss. Boonjira Boontha

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Chemistry

Department of Chemistry

Faculty of Science

Chulalongkorn University

Academic Year 2000

ISBN 974-347-013-1

Thesis Title CYTOTOXICITY OF CHEMICAL CONSTITUENTS OF THE STEM
BARKS OF *Croton oblongifolius* Roxb. FROM MUANG, PRACHUAP
KHIRI KHAEN PROVINCE

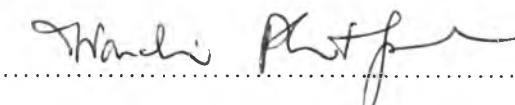
BY Miss. Boonjira Boontha

Field of Study Chemistry

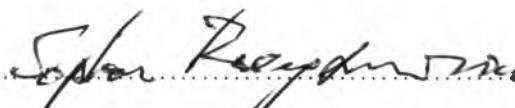
Thesis Advisor Nattaya Ngamrojnavanich, Ph.D.

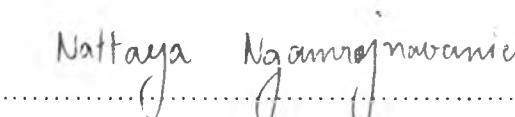
Thesis Co-Advisor Associate Professor Amorn Petsom, Ph.D.

.....
Accepted by the Faculty of Science, Chulalongkorn University in Partial
Fulfillment of the Requirements for the Master's degree.

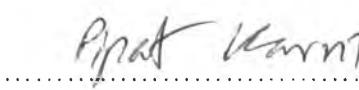
 Dean of Faculty of Science
(Associate Professor Wanchai Phothipichitr, Ph.D.)

Thesis Committee

 Chairman
(Associate Professor Sophon Roengsumran, Ph.D.)

 Thesis Advisor
(Nattaya Ngamrojnavanich, Ph.D.)

 Co-Advisor
(Associate Professor Amorn Petsom, Ph.D.)

 Member
(Associate Professor Pipat Karntiang, Ph.D.)

 Member
(Associate Professor Chaiyo Chaichantipyuth, M. Sc. in Pharm)

บุญจิรา บุญทา : ความเป็นพิษต่อเซลล์มะเร็งของส่วนประกอบทางเคมีจากเปลือกต้นเปล้าใหญ่ (*Croton oblongifolius* Roxb.) จาก อำเภอเมือง จังหวัดประจวบคีรีขันธ์ (CYTOTOXICITY OF CHEMICAL CONSTITUENTS OF THE STEM BARKS OF *Croton oblongifolius* Roxb. FROM MUANG, PRACHUAP KHIRI KHAN PROVINCE

อาจารย์ที่ปรึกษา: ดร. นatasya JAMROJNVAJARATI

อาจารย์ที่ปรึกษาร่วม: รศ. ดร. ออมร เพชรสุม; 118 หน้า. ISBN 974-347-013-1

จากการสกัดเปลือกต้นเปล้าใหญ่ (*Croton oblongifolius* Roxb.) จากอำเภอเมือง จังหวัดประจวบคีรีขันธ์ ด้วยตัวทำละลาย 3 ชนิด คือ เซกเซน, เอทิลอะซิเตท และเมทานอล ตามลำดับ และแยกสารบริสุทธิ์ด้วยวิธีคอลัมน์โครมาโตกราฟีบันชิลิกาเจลได้สาร 6 ชนิด ได้แก่ Trachyloban-19-oic-acid (1) ซึ่งเป็นสารประกอบทรานคิโลบาน, Poilaneic acid (2) ซึ่งเป็นสารประกอบเชนบранอยด์, 12(E),14-labdadiene-7,8-diol (4), 6-acetoxy-12(E),14-labdadiene-7,8-diol (5), 12(E),14-labdadiene-6,7,8-triol (6) ซึ่งเป็นสารประกอบแลปเดน และของผสมของสเตอรอยด์ 2 ชนิด ได้แก่ Stigmasterol and β -sitosterol (3) และเป็นครั้งแรกที่พบสาร 1, 5 และ 6 ในต้นเปล้าใหญ่ และได้สังเคราะห์อนุพันธ์ของสาร 4 โดยปฏิกิริยา acetylation ได้ 7-acetoxy-12,14-labdadiene-8-ol การหาสูตรโครงสร้างของสารต่างๆที่แยกได้โดยอาศัยสมบัติทางกายภาพและทางสเปกโตสโคปี และนำสารที่แยกได้มาทดสอบฤทธิ์ในการยับยั้งเซลล์มะเร็งซึ่งได้แก่ Hep-G2 (ตับ), SW 620 (ลำไส้), Chago (ปอด), Kato-3 (กระเพาะอาหาร) and BT 474 (เต้านม) พบว่าสาร 4 และสาร 6 มีฤทธิ์ในการยับยั้งเซลล์มะเร็งทั้ง 5 ชนิด สาร 5 มีฤทธิ์ในการยับยั้งเซลล์มะเร็งปานกลางกับมะเร็งชนิด Hep-G2, SW 620, Chago และ Kato-3 นอกจากนี้สาร 1 และ 2 มีฤทธิ์ยับยั้งเซลล์มะเร็งชนิด Hep-G2 และ SW 620 เพียงเล็กน้อย

ภาควิชา.....	<i>Chemistry</i>	ลายมือชื่อนิสิต.....	<i>Beonjirat Boontha</i>
สาขาวิชา.....	<i>Organic Chemistry</i>	ลายมือชื่ออาจารย์ที่ปรึกษา.....	<i>ดร. นางสาวนภาพร คงวิจิตร</i>
ปีการศึกษา.....	<i>2006</i>	ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....	<i>ดร. ออมร เพชรสุม</i>

BOONJIRA BOONTHA: CYTOTOXICITY OF CHEMICAL CONSTITUENTS
OF THE STEM BARKS OF *Croton oblongifolius* Roxb. FROM MUANG,
PRACHUAP KHIRI KHAN PROVINCE

THESIS ADVISOR: NATTAYA NGAMROJNAVANICH, Ph.D.

THESIS CO-ADVISOR: Associate Professor AMORN PETSOM, Ph.D.

118 pp. ISBN 974-347-013-1

The stem barks of *Croton oblongifolius* Roxb. were extracted with hexane, ethyl acetate and methanol, respectively. The extracts were separated by column chromatography on silica gel. Six compounds were isolated and identified, namely, Trachyloban-19-oic-acid (1) (trachylobane diterpenoid), Poilaneic acid (2) (cembrane diterpenoid), 12(E),14-labdadiene-7,8-diol (4), 6-acetoxy-12(E),14-labdadiene-7,8-diol (5), 12(E),14-labdadiene-6,7,8-triol (6) (labdane diterpenoid) and a mixed steroid of Stigmasterol and β -sitosterol(3). Moreover, this is the first report of compound 1, 5 and 6 from this plant. Compound 4a was obtained from the acetylation of compound 4, which was assigned as 7-acetoxy-12,14-labdadiene-8-ol. Their physical properties and spectroscopic data identified the structures of the isolate compounds. The compounds were tested for cytotoxicity against cancer cell lines: Hep-G2 (hepatoma), SW 620 (colon), Chago (lung), Kato-3 (gastric) and BT 474 (breast). Compound 4 and compound 6 showed strong activity against all cancer cell lines. Compound 5 and compound 4a showed moderate activity against Hep-G2, SW 620, Chago and Kato-3. However, compound 1 and compound 2 showed only weak activity against Hep-G2 and SW 620.

Department.....

Student's signature

Field of study.....

Advisor's signature Nattaya Ngamrojnavanich

Academic year.....

Co-advisor's signature Amorn Petsom



ACKNOWLEDGEMENT

The author wishes to express her deepest appreciation to Dr. Nattaya Ngamrojnavanich, her major advisor and Associate Professor Dr. Amorn Petsom, her co-advisor for their invaluable suggestions, continuous guidance, encouragement and kindness throughout the course of the research work. She is grateful to Associate Professor Dr. Sophon Roengsumran for his generous guidance and serving as chairman of her thesis committee. Innumerable thanks are extended to Dr. Tirayut Vilaivan for his encouraging guidance during the research work. Grateful acknowledgements are made to Associate Professor Chaiyo Chaichantipyuth and Associate Professor Pipat Karntiang for serving as Examination Committee member and correction of her thesis. Thank were extend to Mrs. Songchan Phuthong, Institute of Biotechnology and Genetic Engineering for cytotoxicity test. She would like to thank the Promotion of Science and Technology (DPST) Project scholarship and Department of Chemistry, Faculty of science and the graduate school, Chulalongkorn University for the financial support during this work.

Finally, she would like to express her deepest gratitude to her parents for encouragement and invaluable advice in everything. In addition, she thanks all her friends, her sisters and her brothers for their friendship and help during her graduate study.

CONTENTS

	Page
ABSTRACT IN THAI.....	iv
ABSTRACT IN ENGLISH	v
ACKNOWLEDGEMENT.....	vi
CONTENTS	vii
LIST OF TABLES	x
LIST OF FIGURES	xii
LIST OF ABBREVIATIONS	xv
CHAPTER I INTRODUCTION	1
CHAPTER II LITERATURE REVIEWS	
2.1 General characteristics of the plants in the Genus Croton.....	4
2.2 Botanical characteristics of <i>C. oblongifolius</i> Roxb.	4
2.3 The chemical constituents of <i>C. oblongifolius</i> Roxb.....	6
2.4 Biological activity of Ditepene compounds.....	12
2.5 Biological activity of diterpene compounds isolated from <i>C. oblongifolius</i> Roxb.	15
2.6 Cytotoxic activity of some compounds of <i>C. oblongifolius</i>	16
2.7 Biosynthesis of diterpenoid compounds	17
2.7.1 Biosynthesis of Labdane compound.....	20
2.7.2 Biosynthesis of Trachylobane compound.....	21
CHAPTER III EXPERIMENTS	
3.1 PLANT MATERIALS.....	22
3.2 INSTRUMENT AND EQUIPMENTS.....	22
3.3 CHEMICAL.....	23
3.4 EXTRACTION AND ISOLATION.....	24

	Page
3.5 SEPARATION OF CRUDE EXTRACTION OF <i>C. oblongifolius</i> Roxb.....	26
3.5.1 Separation of hexane crude extract.....	26
3.5.2 Separation of ethyl acetate crude extract.....	28
3.5.3 Separation of methanol crude extract.....	28
3.6 PURIFICATION AND PHYSICAL PROPERTY OF COMPOUNDS OF HEXANE CRUDE EXTRACT	
3.6.1 Purification and Physical property of Compound <u>1</u>	29
3.6.2 Purification and Physical property of Compound <u>2</u>	31
3.6.3 Purification and Physical property of Mixture <u>3</u>	32
3.6.4 Purification and Physical property of Compound <u>4</u>	33
3.6.5 Purification and Physical property of Compound <u>5</u>	34
3.6.6 Purification and Physical property of Compound <u>6</u>	35
3.6.7 Purification and Physical property of modification of Compound <u>4</u>	36
3.7 PURIFICATION OF THE COMPOUNDS FROM ETHYL ACETATE CRUDE EXTRACT.....	38
3.8 BIOLOGICAL EVALUATION.....	38
CHAPTER IV RESULTS AND DISCUSSION	
4.1 STRUCTURE ELUCIDATION OF THE ISOLATED COMPOUNDS FROM THE STEM BARKS	
OF <i>C. oblongifolius</i> Roxb.	39
4.1.1 Structure elucidation of Compound <u>1</u>	39

	Page
4.1.2 Structure elucidation of Compound <u>2</u>	42
4.1.3 Structure elucidation of Mixture <u>3</u>	45
4.1.4 Structure elucidation of Compound <u>4</u>	49
4.1.5 Structure elucidation of Compound <u>5</u>	53
4.1.6 Structure elucidation of Compound <u>6</u>	56
4.1.7 Structure elucidation of Compound <u>4a</u>	65
4.2 RESULTS OF BIOLOGICAL ACTIVITY.....	69
CHAPTER V CONCLUSION	71
REFFERENCES	73
APPENDIX	78
VITA.....	118

LIST OF TABLES

	Page
Table 1 The chemical constituents of <i>C. oblongifolius</i> Roxb.....	8
Table 2 Plant diterpene biological activity.....	13
Table 3 Cytotoxic activity against cancer cell lines of some diterpene compounds from <i>C. oblongifolius</i>	16
Table 4 The solvent extracts of the stem barks of <i>C. oblongifolius</i> Roxb.....	24
Table 5 The result from column chromatography of hexane crude extract... ..	27
Table 6 Screening test of cytotoxic activity of hexane, ethyl acetate and methanol crude extract of the stem barks of <i>C. oblongifolius</i> from Muang, Prachuab khiri khan province	38
Table 7 The IR absorption bands assignment of Compound <u>1</u>	39
Table 8 Comparison of ^{13}C -NMR data of Compound <u>1</u> with Trachyloban-19-oic acid.....	41
Table 9 The IR absorption band assignment of Compound <u>2</u>	42
Table 10 Comparison of ^{13}C -NMR data of Poilaneic acid with Compound <u>2</u>	44
Table 11 The IR absorption band assignment of Mixture <u>3</u>	45
Table 12 The ^{13}C -NMR chemical shift of Mixture <u>3</u> compared with β -sitosterol	47
Table 13 The IR absorption bands assignment of Compound <u>4</u>	49
Table 14 The ^{13}C -NMR chemical shift of Compound <u>4</u> compared with Nidorellol	52
Table 15 The IR absorption bands assignment of Compound <u>5</u>	53

	Page
Table 16 The ^{13}C -NMR chemical shift of Compound 4 compared with 6-acetoxy-12(E),14-labdadiene-7,8-diol	55
Table 17 The IR absorption bands assignment of Compound <u>6</u>	56
Table 18 The HMQC spectral data of Compound <u>6</u>	58
Table 19 The HMQC, HMBC and COSY spectral data of Compound <u>6</u>	59
Table 20 The ^{13}C -NMR chemical shift of Compound <u>6</u> compared with Crotomachlin.....	61
Table 21 The IR absorption bands assignment of Compound <u>4a</u>	65
Table 22 The ^{13}C -NMR chemical shift of Compound <u>4a</u> compared with 7-acetoxy-12(E),14-labdadiene-8-diol.....	67
Table 23 Cytotoxic activity against 6 cell lines of compounds from <i>C. oblongifolius</i> and Doxorubicin	69
Table 24 The isolated compounds from the stem barks of <i>C. oblongifolius</i> Roxb. and derivative	72

LIST OF FIGURES

Figure	Page
1. <i>Croton oblongifolius</i> Roxb.....	5
2. The structures of diterpenoid compounds from <i>C. oblongifolius</i> Roxb.....	10
3. Biosynthesis of Labdane diterpene.....	20
4. Biosynthesis of Trachylobane diterpene.....	21
5. The acetylation of Compound 4.....	34
6. The structure of Compound 1.....	40
7. The structure of Compound 2.....	43
8. The structure of Stigmasterol and β -sitosterol.....	48
9. The structure of Compound 4.....	51
10. The fragmentation pattern of Compound 4.....	51
11. The structure of Compound 5.....	54
12. The NOESY correlation of Crotomachlin	62
13. The structure of Compound 6.....	62
14. The HMBC correlation of Compound 6.....	63
15. The COSY correlation of Compound 6.....	63
16. The NOESY correlation of Compound 6.....	64
17. The structure of Compound 4a.....	68
18. The IR spectrum of Compound 1.....	79
19. The ^1H -NMR spectrum of Compound 1.....	80
20. The ^{13}C -NMR spectrum of Compound 1.....	81
21. The DEPT 135, 90 ^{13}C -NMR spectrum of Compound 1	82
22. The EIMS spectrum of Compound 1.....	83

Figure	Page
23. The IR spectrum of Compound <u>2</u>	84
24. The ^1H -NMR spectrum of Compound <u>2</u>	85
25. The ^{13}C -NMR spectrum of Compound <u>2</u>	86
26. The DEPT 135, 90 ^{13}C -NMR spectrum of Compound <u>2</u>	87
27. The EIMS spectrum of Compound <u>2</u>	88
28. The IR spectrum of Mixture <u>3</u>	89
29. The ^1H -NMR spectrum of Mixture <u>3</u>	90
30. The ^{13}C -NMR spectrum of Mixture <u>3</u>	91
31. The EIMS spectrum of Mixture <u>3</u>	92
32. The GC spectrum of Mixture <u>3</u>	93
33. The IR spectrum of Compound <u>4</u>	94
34. The ^1H -NMR spectrum of Compound <u>4</u>	95
35. The ^{13}C -NMR spectrum of Compound <u>4</u>	96
36. The DEPT 135,90 ^{13}C -NMR spectrum of Compound <u>4</u>	97
37. The EIMS spectrum of Compound <u>4</u>	98
38. The IR spectrum of Compound <u>5</u>	99
39. The ^1H -NMR spectrum of Compound <u>5</u>	100
40. The ^{13}C -NMR spectrum of Compound <u>5</u>	101
41. The DEPT 135, 90 ^{13}C -NMR spectrum of Compound <u>5</u>	102
42. The EIMS spectrum of Compound <u>5</u>	103
43. The IR spectrum of Compound <u>6</u>	104
44. The ^1H -NMR spectrum of Compound <u>6</u>	105
45. The ^{13}C -NMR spectrum of Compound <u>6</u>	106
46. The DEPT 135, 90 ^{13}C -NMR spectrum of Compound <u>6</u>	107
47. The EI MS spectrum of Compound <u>6</u>	108

Figure	Page
48. The COSY-NMR spectrum of Compound <u>6</u>	109
49. The HMBC-NMR spectrum of Compound <u>6</u>	110
50. The HMQC-NMR spectrum of Compound <u>6</u>	111
51. The NOESY-NMR spectrum of Compound <u>6</u>	112
52. The IR spectrum of Compound <u>4a</u>	113
53. The ¹ H-NMR spectrum of Compound <u>4a</u>	114
54. The ¹³ C-NMR spectrum of Compound <u>4a</u>	115
55. The DEPT 135, 90 ¹³ C-NMR spectrum of Compound <u>4a</u>	116
56. The EIMS spectrum of Compound <u>4a</u>	117

ABBREVIATIONS

br s	=	Broad singlet (for NMR spectra)
c	=	Concentration
⁰ C	=	Degree Celcius
CDCl ₃	=	Deuterated chloroform
CHCl ₃	=	Chloroform
cm	=	Centimeter
¹³ C-NMR	=	Carbon-13 nuclear magnetic resonance
COSY	=	Correlated SpectroscopY
d	=	Doublet (for NMR spectra)
dd	=	Doublet of doublet (for NMR spectra)
ddd	=	Doublet of doublet of doublet (for NMR spectra)
DEPT	=	Distortionless Enhancement by Polarization Transfer
DMSO	=	Dimethyl sulfoxide
δ	=	Chemical Shift
EI MS	=	Electron Impact Mass Spectrum
EtOAc	=	Ethyl acetate
g	=	Gram
¹ H-NMR	=	Proton nuclear magnetic resonance
Hz	=	Hertz
HMBC	=	Heteromolecular Multiple Bond Correlation
HMQC	=	Heteromolecular Multiple Quantum Correlation
IR	=	Infrared spectrum
<i>J</i>	=	Coupling constant

kg	=	Kilogram
L	=	Litre
M ⁺	=	Molecular ion
mg	=	Milligram
MHz	=	Megahertz
ml	=	Millilitre
mm	=	Millimetre
m.p.	=	Melting point
MeOH	=	Methanol
M	=	Molar
m/z	=	Mass to charge ratio
M.W.	=	Molecular weight
MS	=	Mass spectrometry
No.	=	Number
NMR	=	Nuclear Magnetic Resonance
NOESY	=	Nuclear Overhauser Enhancement SpectroscopY
ppm	=	Part per million
q	=	Quartet (for NMR spectra)
s	=	Singlet (for NMR spectra)
t	=	Triplet (for NMR spectra)
TLC	=	Thin layer Chromatography
wt	=	Weight
R _f	=	Retention factor in chromatography