

การแยกและหาสูตรโครงสร้างของสารประกอบจากรากคนทา

(Harrisonia perforata Merr.)



นางสาว มนิตา สกิตม้นาธรรม

ศูนย์วิทยทรัพยากร  
วิทยานิพนธ์ฉบับนี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต  
จุฬาลงกรณ์มหาวิทยาลัย  
ภาควิชาเคมี

บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย

พ.ศ. 2535

ISBN 974-581-470-9

ลิขสิทธิ์ของบัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย

019219

117172597

ISOLATION AND STRUCTURAL DETERMINATION OF COMPOUNDS

FROM ROOTS OF Harrisonia perforata Merr.



Miss Manida Stitmannatham

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science

Department of Chemistry

Graduate School

Chulalongkorn University

1992

ISBN 974-581-470-9



Thesis Title Isolation and Structural Determination of Compounds  
from Roots of Harrisonia perforata Merr.  
By Miss Manida Stitmannaitam  
Department Chemistry  
Thesis Advisor Associate Prof. Sophon Reongsumran, Ph.D.



Accepted by the Graduate School, Chulalongkorn University in  
partial fulfillment of the requirements for the Master's degree.

*Thavorn Vajrabhaya*  
.....Dean of Graduate School  
(Professor Thavorn Vajrabhaya, Ph.D.)

Thesis Committee

*Udom Kokpol*  
.....Chairman  
(Associate Professor Udom Kokpol, Ph.D.)

*Ratana Seangprasertkij*.....Member  
(Assistant Professor Ratana Seangprasertkij, Ph.D.)

*Preecha Ngoviwatthai*.....Member  
(Mr. Preecha Ngoviwatthai, Ph.D.)

*Sophon Roengsumran*.....Member  
(Associate Professor Sophon Roengsumran, Ph.D.)



มณีดา สติemannธรรม : กาวแยกและหาสูตรโครงสร้างของสารประกอบจากราก  
คนทา (*Harrisonia perforata* Merr.) (ISOLATION AND STRUCTURAL  
DETERMINATION OF COMPOUNDS FROM ROOTS OF *Harrisonia perforata*  
Merr.). อ.ที่ปรึกษา : รศ.ดร. โสภณ เรืองสำราญ, 194 หน้า.  
ISBN 974-581-470-9



2-hydroxymethyl-3-methylalloptaeroxylin เป็นสารประกอบชนิดใหม่ที่แยก  
ได้และสารประกอบอื่น ๆ อีกแปดชนิดคือ heteropeucenin-7-methylether, perforatic  
acid, lupeol, คูมารินที่ไม่มีหมู่แทนที่, 5-hydroxy-6,7-dimethoxycoumarin, ของผสม  
ของแอลกอฮอล์ไซตรง (C<sub>31</sub>-C<sub>35</sub>), ของผสมสเตียรอยด์ ( $\beta$ -sitosterol, campesterol  
และ stigmasterol) และ ของผสมของสเตียรอยด์กลัยโคไซด์ ( $\beta$ -sitosteryl-3-O-  
glucopyranoside, chloresteryl-3-O-glucopyranoside, stigmasteryl-3-O-  
glucopyranoside) สารประกอบทั้งหมดนี้แยกได้จากรากคนทา (*Harrisonia perforata*  
Merr.) โดยวิธีการสกัดด้วยตัวทำละลายที่เหมาะสมและเทคนิคทางโครมาโทกราฟี การหา  
สูตรโครงสร้างของสารประกอบที่แยกได้ ทำโดยอาศัยคุณสมบัติทางกายภาพ คุณสมบัติทางเคมี  
และหลักฐานทางสเปกโทรสโกปี นอกจากนี้ยังได้รายงานการวิเคราะห์สิ่งสกปรกในชั้นน้ำพบ  
กรดแอมิโน, เกลือคลอไรด์ และน้ำตาล

ศูนย์วิทยทรัพยากร  
จุฬาลงกรณ์มหาวิทยาลัย

ภาควิชา ..... เคมี  
สาขาวิชา ..... เคมีอินทรีย์  
ปีการศึกษา ..... 2534

ลายมือชื่อนิสิต .....  
ลายมือชื่ออาจารย์ที่ปรึกษา .....  
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....



MANIDA STITMANNAITHAM : ISOLATION AND STRUCTURAL DETERMINATION  
OF COMPOUNDS FROM ROOTS OF *Harrisonia perforata* Merr.  
THESIS ADVISOR : ASSO. PROF. SOPHON ROENGSUMRAN, Ph.D., 194 PP.  
ISBN 974-581-470-9

A novel compound, 2-hydroxymethyl-3-methylalloptaeroxylin and eight known compounds, heteropeucenin-7-methylether, perforatic acid, lupeol, unsubstituted coumarin, 5-hydroxy-6,7-dimethoxycoumarin, saturated long chain aliphatic alcohol (C<sub>31</sub>-C<sub>35</sub>), a mixture of steroids ( $\beta$ -sitosterol, campesterol and stigmasterol) and a mixture of steroidalglycosides ( $\beta$ -sitosteryl-3-O-glucopyranoside, chloresteryl-3-O-glucopyranoside, stigmasteryl-3-O-glucopyranoside) were isolated from the roots of *Harrisonia perforata* Merr. by extracting with suitable solvents and chromatographic techniques. The structures of isolated compounds were established on the basis of physical properties, chemical properties and spectral evidence. This present study also reported the analysis of the aqueous layer extract from which amino acid, chloride salts and sugars were found.



ภาควิชา ..... เคมี  
สาขาวิชา ..... เภสัชกรรม  
ปีการศึกษา ..... 2534

ลายมือชื่อนิติ ..... M Shitmannaitham  
ลายมือชื่ออาจารย์ที่ปรึกษา .....  
ลายมือชื่ออาจารย์ที่ปรึกษา .....



## ACKNOWLEDGEMENT

The author wishes to express her sincere appreciation and heartfelt gratitude to her advisor Associate Professor Dr. Sophon Roengsumran for his valuable guidance, continuous encouragement and helpful suggestion throughout the course of this research. She is grateful to the Rhône-Poulenc Group (Mr. Brian Hughes, MAY & BAKER Thailand) and Professor Jean-Marie Lehn, University of Louis Pasteur (France) for their kindness to grant her a Rhône-Poulenc Professor Lehn scholarship, during 1989-1991. Appreciations are also expressed to Associate Professor Dr. Phichai Tovivich and Assistant Professor Dr. Prapaipit Chamsuksai for their kindness. Thanks are extended to the staff of the Natural Products Unit Cell for their valuable guidance and the Graduate School for financial support in part for this research work. In addition, she wishes to thank her committee, Associate Professor Dr. Udom Kokpol, Assistant Professor Dr. Ratana Seangprasertkij and Dr. Preecha Ngoviwatchai for their comment and suggestion. Besides, she wishes to thank Assistant Professor Dr. Amorn Petsom and Assistant Professor Dr. Somchai Pengpreecha for their valuable guidance about NMR spectra and all of the Scientific and Technology Research Equipment centre's staff, Chulalongkorn University for their helps.

Deep affectionate gratitude is knowledged to her parents , brothers and friends for their moral and spiritual guidance and continuous encouragement throughout the entire study.





## CONTENT

	Page
Abstract in Thai .....	iv
Abstract in English .....	v
Acknowledgement .....	vi
List of Figures .....	x
List of Schemes .....	xiv
List of Tables .....	xv
List of Abbreviations .....	xviii
Chapter	
I INTRODUCTION .....	1
1.1 Botanical Aspects of the Harrisonia .....	1
1.2 Chemical Constituents of Simaroubaceae Studies.	3
1.3 Pharmacological Activities .....	12
1.4 The Goal of this Research .....	13
II EXPERIMENTS AND RESULTS .....	14
2.1 Plant Materials .....	14
2.2 Instruments and Equipment .....	14
2.3 Chemical Reagents .....	17
2.4 Physical Separation Techniques .....	17
2.5 Color Tests .....	21
2.6 Extraction and Initial Fractionations .....	22
2.7 Separation of Fraction Ia and Fraction Ib .....	26
2.8 Separation of Fraction IIa and Fraction IIb ...	31

	Page
2.9 Separation of Fraction IIIa and Fraction IIIb .	38
2.10 Isolation and Purification of Separated Compounds .....	45
2.10.1 Compound ( <u>1</u> ) .....	45
2.10.2 Compound ( <u>2</u> ) .....	47
2.10.3 Compound ( <u>3</u> ) .....	48
2.10.4 Compound ( <u>4</u> ) .....	49
2.10.5 Compound ( <u>5</u> ) .....	54
2.10.6 Compound ( <u>6</u> ) .....	57
2.10.7 Compound ( <u>7</u> ) .....	58
2.10.8 Compound ( <u>8</u> ) .....	59
2.10.9 Compound ( <u>9</u> ) .....	61
2.11 Separation of Water Layer Extracts .....	63
2.11.1 The Inorganic Elemental Analysis .....	63
2.11.2 The Carbohydrate Analysis .....	63
2.11.3 The Amino Acid Analysis .....	65
III RESULTS AND DISCUSSION .....	66
3.1 Structural Elucidation of the Isolated Compounds .....	66
3.1.1 Compound ( <u>1</u> ) .....	66
3.1.2 Compound ( <u>2</u> ) .....	73
3.1.3 Compound ( <u>3</u> ) .....	77
3.1.4 Compound ( <u>4</u> ) .....	82
3.1.5 Compound ( <u>5</u> ) .....	85



	Page
3.1.6 Compound (6) .....	92
3.1.7 Compound (7) .....	95
3.1.8 Compound (8) .....	101
3.1.9 Compound (9) .....	104
3.2 Biological Activity and Utilization of Isolated Compounds .....	111
3.2.1 Compound (1) .....	111
3.2.2 Compound (2) .....	111
3.2.3 Compound (3) .....	112
3.2.4 Compound (4) .....	112
3.2.5 Compound (5) .....	112
3.2.6 Compound (6) .....	113
3.2.7 Compound (7) .....	113
3.2.8 Compound (8) .....	114
3.2.9 Compound (9) .....	114
3.3 The Separation Compounds from <i>Harrisonia</i> <i>perforata</i> Merr. ....	115
IV CONCLUSION .....	117
REFERENCES .....	123
VITA .....	194



## List of Figures

Figure		Page
1	The IR spectrum of Compound ( <u>1</u> ) .....	133
2	The $^1\text{H}$ NMR spectrum of Compound ( <u>1</u> ) .....	134
3	The $^{13}\text{C}$ NMR spectrum of Compound ( <u>1</u> ) .....	135
4	The mass spectrum of Compound ( <u>1</u> ) .....	136
5	The GLC analysis results of Compound ( <u>1</u> ) .....	137
6	The IR spectrum of Compound ( <u>2</u> ) .....	138
7	The $^1\text{H}$ NMR spectrum of Compound ( <u>2</u> ) .....	139
8	The $^{13}\text{C}$ NMR spectrum of Compound ( <u>2</u> ) .....	140
9	The UV spectrum of Compound ( <u>2</u> ) .....	141
10	The mass spectrum of Compound ( <u>2</u> ) .....	142
11	The IR spectrum of Compound ( <u>3</u> ) .....	143
12	The $^1\text{H}$ NMR spectrum of Compound ( <u>3</u> ) .....	144
13	The $^{13}\text{C}$ NMR spectrum of Compound ( <u>3</u> ) .....	145
14	The mass spectrum of Compound ( <u>3</u> ) .....	146
15	The IR spectrum of Compound ( <u>4</u> ) .....	147
16	The $^1\text{H}$ NMR spectrum of Compound ( <u>4</u> ) .....	148
17	The $^{13}\text{C}$ NMR spectrum of Compound ( <u>4</u> ) .....	149
18	The mass spectrum of Compound ( <u>4</u> ) .....	150
19	The IR spectrum of Compound ( <u>4a</u> ) .....	151
20	The GLC analysis results of Compound ( <u>4b</u> ) .....	152
21	The IR spectrum of Compound ( <u>4a</u> ) acetate .....	153
22	The IR spectrum of Compound ( <u>5</u> ) .....	154
23	The IR spectrum of Compound ( <u>5</u> ) acetate .....	155
24	The $^1\text{H}$ NMR spectrum of Compound ( <u>5</u> ) acetate .....	156



Figure	Page
25 The $^{13}\text{C}$ NMR spectrum of Compound (5) acetate .....	157
26 The The mass spectrum of Compound (5) acetate .....	158
27 The GLC analysis results of Compound (5) acetate .....	159
28 The IR spectrum of hydrolysis product of Compound (5) acetate (an alcoholic part) .....	160
29 The $^1\text{H}$ NMR spectrum of hydrolysis product of Compound (5) acetate (an alcoholic part) .....	161
30 A) The $^{13}\text{C}$ NMR spectrum of hydrolysis product of Compound (5) acetate (an alcoholic part) .....	162
30 B) The $^{13}\text{C}$ NMR spectrum DEPT 90 of hydrolysis product of Compound (5) acetate (an alcoholic part) .....	163
30 C) The $^{13}\text{C}$ NMR spectrum DEPT 135 of hydrolysis product of Compound (5) acetate (an alcoholic part) .....	164
31 The UV spectrum of hydrolysis product of Compound (5) acetate (an alcoholic part) .....	165
32 The mass spectrum of hydrolysis product of Compound (5) acetate (an alcoholic part) .....	166
33 The GLC analysis results of hydrolysis product of Compound (5) acetate (an alcoholic part) .....	167
34 The IR spectrum of Compound (6) .....	168
35 The $^1\text{H}$ NMR spectrum of Compound (6) .....	169
36 A) The $^{13}\text{C}$ NMR spectrum of Compound (6) .....	170
B) The $^{13}\text{C}$ NMR spectrum DEPT 90 of Compound (6) .....	171
C) The $^{13}\text{C}$ NMR spectrum DEPT 135 of Compound (6) .....	171
37 The mass spectrum of Compound (6) .....	172



Figure	Page
38 The IR spectrum of Compound (7) .....	173
39 The $^1\text{H}$ NMR spectrum of Compound (7) .....	174
40 A) The $^{13}\text{C}$ NMR spectrum of Compound (7) .....	175
B) The $^{13}\text{C}$ NMR spectrum DEPT 90 of Compound (7) .....	176
C) The $^{13}\text{C}$ NMR spectrum DEPT 135 of Compound (7) .....	177
41 The mass spectrum of Compound (7) .....	178
42 The IR spectrum of Compound (8) .....	179
43 The $^1\text{H}$ NMR spectrum of Compound (8) .....	180
44 The $^{13}\text{C}$ NMR spectrum of Compound (8) .....	181
45 The mass spectrum of Compound (8) .....	182
46 The GLC analysis results of Compound (8) .....	183
47 The standard correlation curve of Compound (8) .....	184
48 The IR spectrum of Compound (8) acetate .....	185
49 The IR spectrum of Compound (9) .....	186
50 The $^1\text{H}$ NMR spectrum of Compound (9) .....	187
51 A) The $^{13}\text{C}$ NMR spectrum of Compound (9) .....	188
B) The $^{13}\text{C}$ NMR spectrum DEPT 135 of Compound (9) .....	189
52 The mass spectrum of Compound (9) .....	190
53 The inorganic elemental analysis of water crude extract by Energy Dispersive X-Ray Fluorescence Spectrometer .....	191
54 The carbohydrate analysis of water extract by High Performance Liquid Chromatography .....	192
55 Amino acid analysis of water crude extract by Amino Acid Analyzer .....	193



Figure	Page
56 The structure of compounds that were isolated from roots of <i>Harrisonia perforata</i> Merr. ....	120



ศูนย์วิทยทรัพยากร  
จุฬาลงกรณ์มหาวิทยาลัย



## List of Schemes

Scheme	Page
2.1 Extraction procedure of dry roots of <i>Harrisonia perforata</i> Meer. ....	24
3.1 The possible mass fragmentation pattern of Compound (1) .....	72
3.2 The possible mass fragmentation pattern of Compound (2) .....	76
3.3 The possible mass fragmentation pattern of Compound (3) .....	81
3.4 The possible mass fragmentation pattern of Compound (5) .....	91
3.5 The possible mass fragmentation pattern of Compound (6) .....	95
3.6 The possible mass fragmentation pattern of Compound (7) .....	100
3.7 The possible mass fragmentation pattern of Compound (9) .....	109
3.8 The isolation compounds from roots of <i>Harrisonia perforata</i> Merr. ....	116



## List of Tables

Table	Page
1.1 Some compounds were isolated from plants in Simaroubaceae family .....	8
1.2 The utility as herbs from Simaroubaceae family .....	12
1.3 The utility of <i>Harrisonia perforata</i> Merr. ....	13
2.1 The results of extraction the sun-dried roots of <i>Harrisonia perforata</i> Meer. ....	25
2.2 The results of Fraction Ia separated on aluminium oxide quick column .....	27
2.3 The results of Fraction Ia separated on silica gel column .....	28
2.4 The results of Fraction Ia separated on aluminium oxide column .....	29
2.5 The results of Fraction Ib separated on silica gel column .....	30
2.6 The results of Fraction IIb separated on aluminium oxide column .....	32
2.7 The results of Fraction IIb separated on silica gel column .....	34
2.8 The results of Fraction IIb separated on aluminium oxide column .....	35
2.9 The results of Fraction IIb separated on silica gel column .....	37
2.10 The results of Fraction IIb separated on aluminium column .....	39



Table	Page
2.11 The results of Fraction IIIa separated on aluminium oxide column .....	40
2.12 The results of Fraction No. 31-36 separated on aluminium oxide column .....	41
2.13 The results of Fraction IIIb separated on silica gel column .....	42
2.14 The results of Fraction IIIb separated on aluminium oxide column .....	43
2.15 The results of Fraction No.31-42 separated on aluminium oxide column .....	44
2.16 The retention times of various peaks from the gas chromatogram of Compound (1) compared with steroids standard .....	46
2.17 The retention times of various peaks from gas chromatogram of Compound (4a) compared with steroids standard .....	52
2.18 The comparison data between standard sugars and crude extract from water layer .....	64
3.1 The IR absorption band assignments of Compound (1) ....	67
3.2 The <sup>13</sup> C NMR chemical shift of $\beta$ -sitosterol, stigmasterol and Compound (1) .....	68
3.3 The comparison of GLC retention times between Compound (1) and authentic steroids .....	71
3.4 The IR absorption band assignments of Compound (2) ....	74



Table	Page
3.5 The $^1\text{H}$ NMR chemical shifts of heteropeucenin-7-methyl ether and Compound (2) .....	75
3.6 The IR absorption band assignments of Compound (3) ....	78
3.7 The $^{13}\text{C}$ NMR chemical shifts of Compound (3) and perforatic acid .....	80
3.8 The IR absorption band assignments of Compound (4) ....	83
3.9 The IR absorption band assignments of Compound (5) ....	86
3.10 The $^1\text{H}$ NMR chemical shift assignments of Compound (5) and Compound (5) acetate .....	88
3.11 The comparison data among moretenol, 3-epimoretenol, lupeol , 3-epilupeol and Compound (5) .....	90
3.12 The IR absorption band assignments of Compound (6) ....	93
3.13 The $^{13}\text{C}$ NMR chemical shift tentative assignments of Compound (6) compared with unsubstituted coumarin .....	94
3.14 The IR absorption band assignments of Compound (7) ....	96
3.15 The $^{13}\text{C}$ NMR chemical shift of unsubstituted coumarin and the observed chemical shift of Compound (7) and the calculated chemical shift of Compound (7) .....	99
3.16 The IR absorption band assignments of Compound (8) ....	102
3.17 The IR absorption band assignments of Compound (9) ....	105
3.18 The $^{13}\text{C}$ NMR chemical shift of Compound (9) and 2-hydroxymethylalloptaeroxylin .....	107



## List of Abbreviation

b	broad	MW	molecular weight
°C	degree Celsius	M <sup>+</sup>	molecular ion in
ca.	ciaca		mass spectrum
cpd.	compound	nm	nanometre
cm <sup>-1</sup>	unit of wavenumber	PC	paper chromatography
d	doublet (NMR)	ppm.	part per million
dd	double doublet	Rf	rate of flow in
DMSO	dimethyl sulfoxide		chromatography
2,4-DNP	2,4-dinitrophenyl	s	singlet (NMR)
	hydrazine	s	sharp (IR)
g	gram (s)	TLC	thin-layer
GLC	gas liquid chromatography		chromatography
hrs	hours	wt.	weight
HPLC	high performance liquid		
	chromatography		
Hz	Hertz		
J	coupling constant		
kg	kilogram (s)		
l	litre (s)		
m	multiplet (NMR)		
m/e	mass to charge ratio		
mg.	milligram (s)		
ml.	millilitre (s)		
m.p.	melting point		