

องค์ประกอบทางเคมีของดอกลำดวน

นาย สุภชัย ตยวรนนท์



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเกสัชศาสตรมหาบัณฑิต

สาขาวิชาเภสัชเวท ภาควิชาเภสัชเวท

บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2541

ISBN 974-331-117-3

ลิขสิทธิ์ของบัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย

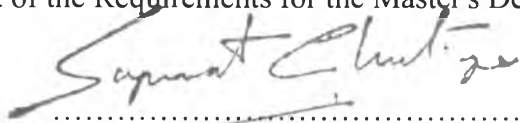
CHEMICAL CONSTITUENTS
OF
MELODORUM FRUTICOSUM FLOWERS

Mr. Suppachai Tiyaworanan

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy
Department of Pharmacognosy
Graduate School
Chulalongkorn University
Academic Year 1998
ISBN 974-331-117-3

Thesis Title Chemical Constituents of *Melodorum fruticosum* Flowers
By Mr. Suppachai Tiyaworanan
Department Pharmacognosy
Thesis Advisor Associate Professor Chaiyo Chaichantipyuth

Accepted by the Graduate School, Chulalongkorn University in Partial
Fulfillment of the Requirements for the Master's Degree



.....Dean of Graduate School
(Professor Supawat Chutivongse, M.D.)

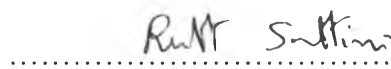
Thesis committee

.....Chairman

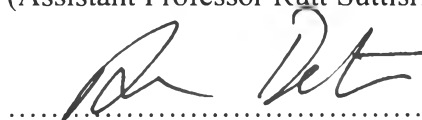
(Associate Professor Kittisak Likhitwitayawuid, Ph.D.)

.....Thesis Advisor

(Associate Professor Chaiyo Chaichantipyuth, M.Sc.)

.....Member

(Assistant Professor Rutt Suttisri, Ph.D.)

.....Member

(Associate Professor Amorn Petsom, Ph.D.)

ศุภชัย ดิยวรรณันท์ : องค์ประกอบทางเคมีของดอกกลาดวน (CHEMICAL CONSTITUENTS OF MELODORUM FRUTICOSUM FLOWERS) อาจารย์ที่ปรึกษา : รศ. ชัยโย ชัยชาญทิพยุทธ, 98 หน้า. ISBN 974-331-117-3

การศึกษาองค์ประกอบทางเคมีของดอกกลาดวน (วงศ์ Annonaceae) สามารถแยกสารบริสุทธิ์ได้ 2 กลุ่ม เป็นสารในกลุ่มเฮปทีน 4 ชนิด และสารกลุ่มฟลาโวน 2 ชนิด และสามารถพิสูจน์สูตรโครงสร้างทางเคมีของสารบริสุทธิ์ที่แยกได้โดยวิธีการทางสเปกโตรสโคปีชนิดต่าง ๆ และเปรียบเทียบข้อมูลที่ได้กับสารที่มีการรายงานในอดีต พบว่าสารบริสุทธิ์ที่แยกได้ 3 ชนิด เป็นอนุพันธ์ใหม่ของสารกลุ่มเฮปทีนที่ยังไม่มีการรายงาน มีชื่อทางเคมีว่า (4Z)-7-benzoyloxy-2,4-heptadiene-6-one-4-olide, (4E)-7-benzoyloxy-2,4-heptadiene-6-one-4-olide, และ (E)-7-benzoyloxy-4-hydroxy-1-methoxy-2,4-heptadiene-1,6-dione และได้ให้ชื่อสามัญแก่สารที่แยกได้นี้ว่า melodorinone-A, melodorinone-B, และ tautomelodienone ตามลำดับ สำหรับสารบริสุทธิ์ที่แยกได้อีก 3 ชนิด เมื่อพิสูจน์สูตรโครงสร้างทางเคมีแล้ว พบว่าเป็นสารที่มีชื่อสามัญว่า acetyl melodorinol, chrysin, และ 5,7-dimethoxyflavone และเมื่อทำการทดสอบความเป็นพิษต่อเซลล์ พบว่าสาร tautomelodienone มีฤทธิ์ต้านเซลล์มะเร็งเม็ดเลือดขาว P388 อย่างน่าสนใจ โดยมีค่า IC_{50} อยู่ที่ 0.60 ไมโครกรัม/มิลลิลิตร

ภาควิชา เกษัชเวช
สาขาวิชา เกษัชเวช
ปีการศึกษา 2541

ลายมือชื่อนิติ
ลายมือชื่ออาจารย์ที่ปรึกษา ชัยโย ชัยชาญทิพยุทธ
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

#4076528833 : MAJOR PHARMACOGNOSY

KEY WORD: HEPTENE / *Melodorum fruticosum* / MELODORINONE-A / MELODORINONE-B / TAUTOMELODIENONE

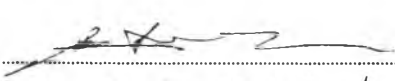
SUPPACHAI TIYAWORANAN : CHEMICAL CONSTITUENTS OF *MELODORUM FRUTICOSUM* FLOWERS. THESIS ADVISOR : ASSOCIATE PROFESSOR CHAIYO CHAICHANTIPYUTH, M.Sc. in Pharm. 98 pp. ISBN 974-331-117-3

This research on the chemical constituents of *Melodorum fruticosum* Lour. flowers (Annonaceae) led to the isolation of four heptene derivatives and two flavones. The structure identification and structure elucidation of these compounds were based on the data from various spectroscopic techniques and comparison with reported data. Three novel derivatives of heptenes were assigned the chemical structures (4*Z*)-7-benzoyloxy-2,4-heptadiene-6-one-4-olide, (4*E*)-7-benzoyloxy-2,4-heptadiene-6-one-4-olide, and (*E*)-7-benzoyloxy-4-hydroxy-1-methoxy-2,4-heptadiene-1,6-dione, and were given the trivial names melodorinone-A, melodorinone-B, and tautomelodienone, respectively. The other three are the known compounds acetylmelodorinol, chrysin, and 5,7-dimethoxyflavone. Tautomelodienone was found to exhibit interesting cytotoxic activity against P388 cell line, with an IC₅₀ value of 0.60 µg/ml.

ภาควิชา..... เกษษเวท

สาขาวิชา..... เกษษเวท

ปีการศึกษา..... 2541

ลายมือชื่อผู้ผลิต..... 

ลายมือชื่ออาจารย์ที่ปรึกษา..... *Chaiyo Chaichantipyuth*

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....



ACKNOWLEDGEMENTS

The author wishes to express his deepest gratitude to his thesis advisor, Associate Professor Chaiyo Chaichantipyuth, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for his valuable advice, guidance, suggestion and encouragement throughout the course of this study.

The author would like to acknowledge his sincere thanks to his thesis committee, for valuable suggestion and encouragement.

The author would like to acknowledge his grateful thanks to Dr. Khanit Suwanborirux, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for his valuable suggestion and helpful in spectroscopic discussion.

The author wishes to express his sincere thanks to Associate Professor Dr. Amorn Petsom, Department of Chemistry, Faculty of Science, Chulalongkorn University, for his meaningful concern and encouragement.

The author would like to thank the University Development Commission (UDC) and the Faculty of Pharmaceutical Sciences, Khon Kaen University, for granting the scholarship throughout the course of his study.

The author would also like to thank the Graduate School of Chulalongkorn University for granting partial financial support to conduct this investigation.

The author would like to thank all his teachers and his friends for their contribution and friendship

Finally, the author wishes to express his infinite gratitude to his family for their love, concern and encouragement.

CONTENTS

	Page
ABSTRACT (Thai)	iv
ABSTRACT (English)	v
ACKNOWLEDGEMENTS.....	vi
CONTENTS	vii
LIST OF TABLES.....	ix
LIST OF FIGURES	x
LIST OF ABBREVIATIONS	xii
CHAPTER	
I. INTRODUCTION.....	1
II. HISTORICAL	4
III. EXPERIMENTAL	
1. Source of Plant Material.....	9
2. General Techniques	
2.1. Analytical Thin-layer Chromatography (TLC).....	9
2.2. Column Chromatography (CC)	
2.2.1. Flash Column Chromatography	9
2.2.2. Gel Filtration Chromatography	10
2.3. Preparative, Centrifugal, Thin Layer Chromatography	10
2.4. Spectroscopic Techniques	
2.4.1. Ultraviolet (UV) Absorption Spectra	11
2.4.2. Infrared (IR) Absorption Spectra	11
2.4.3. Mass Spectra (MS).....	11
2.4.4. Nuclear Magnetic Resonance (NMR) Spectra	11
2.4.5. Circular Dichroism Spectra.....	12
2.5. Physical Properties Measurement Apparatus	
2.5.1. Melting Point.....	12
2.5.2. Optical Rotation	12

2.5.3. Elemental Components	12
2.6. Solvents.....	12
3. Bioassay.....	13
4. Extraction and Isolation.....	13
Physical and Spectral Data of Isolated Compounds	
1. Compound MF-1	18
2. Compound MF-2.....	18
3. Compound MF-3	18
4. Compound MF-4	19
5. Compound MF-5	19
6. Compound MF-6	19
IV. RESULTS AND DISCUSSION	
Structure Determination of Isolated Compounds	
1. Structure Elucidation of Compound MF-1	20
2. Structure Elucidation of Compound MF-2	23
3. Identification of Compound MF-3	24
4. Identification of Compound MF-4	26
5. Structure Elucidation of Compound MF-5	28
6. Identification of Compound MF-6.....	31
V. CONCLUSION.....	35
REFERENCES	83
VITA.....	85

LIST OF TABLES

Table	Page
1. Chemical constituents of <i>Melodorum fruticosum</i> Lour.	4
2. ¹³ C NMR (75 MHz in CDCl ₃), ¹ H NMR (300 MHz in CDCl ₃), C-H COSY (75 MHz in CDCl ₃) and COLOC spectral data of compound MF-1	22
3. ¹³ C chemical shifts of benzoyl moiety of compound MF-1 compared with the data of acetylmelodorinol	22
4. ¹³ C NMR (75 MHz in CDCl ₃), ¹ H NMR (300 MHz in CDCl ₃), C-H COSY (75 MHz in CDCl ₃) and COLOC spectral data of compound MF-2.....	24
5. ¹ H NMR (300 MHz in DMSO- <i>d</i> ₆) spectral data and C-H COSY (75 MHz in DMSO- <i>d</i> ₆) data of compound MF-3	25
6. NMR spectral data comparing of compound MF-3 with chrysin.....	26
7. NMR spectral data comparison of compound MF-4 with 5,7-dimethoxyflavone.....	27
8. ¹³ C NMR (75 MHz in CDCl ₃), ¹ H NMR (300 MHz in CDCl ₃), C-H COSY (75 MHz in CDCl ₃) and COLOC spectral data of compound MF-4	28
9. ¹³ C NMR (75 MHz in CDCl ₃), ¹ H NMR (300 MHz in CDCl ₃), C-H COSY (75 MHz in CDCl ₃) and COLOC spectral data of compound MF-5.....	30
10. ¹³ C NMR (75 MHz in CDCl ₃), ¹ H NMR (300 MHz in CDCl ₃), C-H COSY (75 MHz in CDCl ₃) and COLOC spectral data of compound MF-6.....	32
11. NMR spectral data of compound MF-6 compared with acetylmelodorinol	33
12. Cytotoxicity data of Compound MF-1, MF-2, and MF-5	34

LIST OF FIGURES

Figure	Page
1. <i>Melodorum fruticosum</i> Lour.....	3
2. Structure of chemical component of <i>Melodorum fruticosum</i>	6
3. Extraction scheme of <i>Melodorum fruticosum</i> flowers.....	16
4. Isolation scheme of 70% aqueous acetone extract	16
5. Isolation scheme of ethyl acetate extract.....	17
6. Isolation scheme of petroleum ether extract.....	17
7. 300 MHz Proton NMR Spectrum of Compound MF-1.....	36
8. 75 MHz Carbon-13 NMR Spectrum of Compound MF-1	37
9. DEPT-90 and DEPT-135 Spectra of Compound MF-1.....	38
10. H-H COSY NMR Spectrum of Compound MF-1	39
11. C-H COSY NMR Spectrum of Compound MF-1	40
12. COLOC NMR Spectrum of Compound MF-1	41
13. NOE Difference Spectra of Compound MF-1	42
14. 30 eV CI Mass Spectrum of Compound MF-1.....	43
15. 70 eV EI Mass Spectrum of Compound MF-1	44
16. Infrared Spectrum of Compound MF-1	45
17. 300 MHz Proton NMR Spectrum of Compound MF-2.....	46
18. 75 MHz Carbon-13 NMR Spectrum of Compound MF-2	47
19. H-H COSY NMR Spectrum of Compound MF-2.....	48
20. C-H COSY NMR Spectrum of Compound MF-2	49
21. COLOC NMR Spectrum of Compound MF-2	50
22. NOE Difference Spectra of Compound MF-2.....	51
23. 30 eV CI Mass Spectrum of Compound MF-2.....	52
24. 70 eV EI Mass Spectrum of Compound MF-2.....	53
25. Infrared Spectrum of Compound MF-2.....	54
26. 300 MHz Proton NMR Spectrum of Compound MF-3.....	55
27. 75 MHz Carbon-13 NMR Spectrum of Compound MF-3	56
28. H-H COSY NMR Spectrum of Compound MF-3.....	57

29. C-H COSY NMR Spectrum of Compound MF-3	58
30. 70 eV EI Mass Spectrum of Compound MF-3	59
31. Infrared Spectrum of Compound MF-3	60
32. 300 MHz Proton NMR Spectrum of Compound MF-4.....	61
33. 75 MHz Carbon-13 NMR Spectrum of Compound MF-4	62
34. H-H COSY NMR Spectrum of Compound MF-4.....	63
35. C-H COSY NMR Spectrum of Compound MF-4.....	64
36. COLOC NMR Spectrum of Compound MF-4	65
37. 70 eV EI Mass Spectrum of Compound MF-4.....	66
38. Infrared Spectrum of Compound MF-4.....	67
39. 300 MHz Proton NMR Spectrum of Compound MF-5.....	68
40. 75 MHz Carbon-13 NMR Spectrum of Compound MF-5	69
41. C-H COSY NMR Spectrum of Compound MF-5	70
42. COLOC NMR Spectrum of Compound MF-5	71
43. 70 eV EI Mass Spectrum of Compound MF-5.....	72
44. Infrared Spectrum of Compound MF-5.....	73
45. 300 MHz Proton NMR Spectrum of Compound MF-6.....	74
46. 75 MHz Carbon-13 NMR Spectrum of Compound MF-6	75
47. H-H COSY NMR Spectrum of Compound MF-6.....	76
48. C-H COSY NMR Spectrum of Compound MF-6.....	77
49. COLOC NMR Spectrum of Compound MF-6	78
50. NOE Difference Spectra of Compound MF-6.....	79
51. 70 eV EI Mass Spectrum of Compound MF-6.....	80
52. Infrared Spectrum of Compound MF-6.....	81
53. Circular Dichroism Spectrum of Compound MF-6.....	82

ABBREVIATIONS

$[\alpha]_{589}^{25}$	=	Specific Rotation at 25°C, 589 nm
br	=	Broad
c	=	Concentration
calcd.	=	Calculation
CD	=	Circular Dichroism
CI	=	Chemical Ionization
°C	=	Degree Celsius
CDCl ₃	=	Deuterated chloroform
CHCl ₃	=	Chloroform
cm	=	Centimeter
COLOC	=	Heteronuclear Correlation Long-Range Coupling
¹³ C NMR	=	Carbon-13 Nuclear Magnetic Resonance
H-H COSY	=	Homonuclear (Proton-Proton) Correlation Spectroscopy
2D	=	Two Dimensional
d	=	doublet
dd	=	doublet of doublets
ddd	=	doublet of doublets of doublets
DEPT	=	Distortionless Enhancement by Polarization Transfer
DMSO- <i>d</i> ₆	=	Deuterated Dimethylsulfoxide
δ	=	Chemical Shift
EI	=	Electron Impact
g	=	Gram
¹ H NMR	=	Proton Nuclear Magnetic Resonance
Hz	=	Hertz
IR	=	Infrared Spectrum
<i>J</i>	=	Coupling Constant
Kg	=	Kilogram
L	=	Liter
λ _{max}	=	Wavelength at maximal absorption

ϵ	=	Molar Absorptivity
m	=	Multiplet
mg	=	Milligram
ml	=	Milliliter
MS	=	Mass Spectroscopy
m/z	=	mass-to-charge ratio
M^+	=	Molecular Ion
MH^+	=	Quasimolecular Ion
No.	=	Number
nm	=	Nanometer
NMR	=	Nuclear Magnetic Resonance
NOE	=	Nuclear Overhauser Effect
ppm	=	part per million
ν_{\max}	=	Wave number at maximal absorption
s	=	Singlet
t	=	triplet
TLC	=	Thin Layer Chromatography
UV/vis	=	Ultraviolet and Visible Spectrophotometry
$[\theta]^{25}$	=	Molar ellipticity at 25°C