

สารค้านการกินของแมลงจากไร่落ちแหงแวง

Trigonostemon reidioides Craib

นายธีรุตติ หัวอ่านวบพร



สถาบันวิทยบริการ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาเคมี ภาควิชาเคมี

บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2541

ISBN 974-331-830-5

ลิบสิทธิ์ของบัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย

INSECT ANTIFEEDANTS FROM THE ROOTS OF
Trigonostemon reidioides Craib

Mr. THEERAWUT WANG-AMNAUPORN

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Chemistry

Department of Chemistry

Graduate School

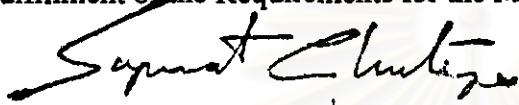
Chulalongkorn University

Academic Year 1998

ISBN 974-331-830-5

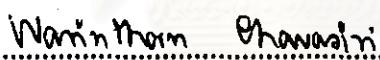
Thesis Title Insect Antifeedants from the Roots of
Trigonostemon reidioides Craib.
By Mr. Theerawut Wang-amnauyuporn
Department Chemistry
Thesis Advisor Assistant Professor Warinthorn Chavasiri, Ph.D.

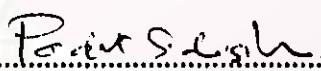
Accepted by the Graduate School, Chulalongkorn University in Partial
Fulfillment of the Requirements for the Master's Degree.


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

Thesis Committee:


..... Chairman
(Associate Professor Siri Varothai, Ph.D.)


..... Thesis Advisor
(Assistant Professor Warinthorn Chavasiri, Ph.D.)


..... Member
(Professor Padet Sidisunthorn, Ph.D.)


..... Member
(Associate Professor Udom Kokpol, Ph.D.)

ธีรุณี หวังอิ้านวยพร : สารต้านการกินของแมลงจากรากไถคหบงແಡ (INSECT ANTIFEEEDANTS FROM THE ROOTS OF *Trigonostemon reidiooides* Craib.)
อ.ที่ปรึกษา : ผศ.ดร. วนิช ชาคริ; 112 หน้า. ISBN 974-331-830-5

ผลการทดลองฤทธิ์ต้านการกินของแมลงเมืองด้วยสั่งสกัดเอกเซนและไอลคตอลไมเนทนากรากไถคหบงແດງ กับหนอนผึ้งกินไข่ผึ้งขนาดใหญ่, *Galleria mellonella*. ปรากฏว่า มีฤทธิ์ต้านการกินในระดับสูง เมื่อนำสั่งสกัดทั้งสองมาแยกกันแล้ว ทราบมาโดยรวมได้ 9 ชนิด โดยยาหัขสมบัติทางกายภาพและหลักฐานทางสถาปัตย์ไทรโยค พิสูจน์ทราบได้ว่าสารทั้ง 9 ชนิด คือ ของผสมของสเตอรอยด์เช่นเชสเตอร์อล (β -sitosterol, stigmasterol และ campesterol), acetyl aleuritolic acid, 5α -stigmastane-3,6-dione, Trigonostemone, 5-hydroxy-6,7-dimethoxy coumarin, ของผสมเช่นเชสเตอร์อล แต่ของผสมสเตอรอยด์ไกโตกไซด์ จำกสารทั้งหมดที่แยกได้พบว่า acetyl aleuritolic acid และของผสมสเตอรอยด์ เป็นสารหลักที่มีฤทธิ์ต้านการกินของแมลงในระดับสูง นอกจากนี้สามารถแยกสารจากสั่งสกัดเอทธิล แอดซีเตตได้อีก 2 ชนิด คือ สารโครงสร้างของสารทั้งสองพิสูจน์ทราบได้ว่าเป็น 5-hydroxy-6,7-dimethoxy coumarin กับ 5,7-dihydroxy-6-methoxy coumarin สารชนิดหลังพบว่าเป็นภูมิรินท์พบในธรรมชาติชนิดใหม่

สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย

ภาควิชา..... (1ม.)
สาขาวิชา..... (1ม.)
ปีการศึกษา 2541

ตามมือชื่อนักศึกษา ธีรุณี วงศ์ตานะรุษฯ
ตามมือชื่ออาจารย์ที่ปรึกษา วนิช ชาคริ
ตามมือชื่ออาจารย์ที่ปรึกษาร่วม

C825325 CHEMISTRY

KEY WORD: ^{MAJOR} *Trigonostemon reidioides* Craib. / INSECT ANTIFEEDANT / *Galleria mellonella*

THEERAWUT WANG-AMNAUYPORN : INSECT ANTIFEEDANTS FROM
THE ROOTS OF *Trigonostemon reidioides* Craib. ADVISOR : ASSIST. PROF.
WARINTHORN CHAVASIRI, Ph.D. 112 PP. ISBN 974-331-830-5

The preliminary results of insect antifeedant activity tests of crude hexane and dichloromethane extracts from the roots of *Trigonostemon reidioides* Craib. revealed that they displayed high activity. The separation of both crude extracts by column chromatography led to the isolation of 9 substances. By means of physical properties and spectroscopic data, they were characterized as a mixture of steroidal esters, a mixture of long chain acids, a mixture of steroids, acetyl aleuritolic acid, Trigonostemone, 5α -stigmastane-3,6-dione, 5-hydroxy-6,7-dimethoxy coumarin, a mixture of long chain amides, and a mixture of steroidal glycosides. From all separated substances, acetyl aleuritolic acids and a mixture of steroids are two active principles that showed highly active insect antifeedant activity. Furthermore, other two compounds were also isolated from ethyl acetate crude extract. Their structures were identified as 5-hydroxy-6,7-dimethoxy coumarin and 5,7-dihydroxy-6-methoxy coumarin. The latter was found to be a new naturally occurring coumarin.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

ภาควิชา.....เคมี
สาขาวิชา.....เคมี
ปีการศึกษา..... 2541

ลายมือชื่อนิสิต..... ตั้ง วงศ์ วงศ์ วงศ์
ลายมือชื่ออาจารย์ที่ปรึกษา..... ดร. พ. พ.
ลายมือชื่ออาจารย์ที่ปรึกษาawan



ACKNOWLEDGEMENT

On the endeavor to create a piece of work, it begins from the scattering of ideas until to be a pattern, having a framework and content to be called a thesis. No matter how value of this work is evaluated but for thesis owner it is an assemblage of the collaboration, helping and friendship from many people and the symbol of gaining the meaningful experience.

Over of all, a person who created chemical knowledge paradigm and research thinking to me is my academic advisor Assistant Professor Dr. Warinthorn Chavasiri. In his advisory status, he helps to activate the potentiality of one student as seen in this thesis and gives me a chance to present free opinion and discussion during the preparation of this thesis that I owe him a great deal of thank for his kindness, concern and attention.

Appreciation is also expressed to the Faculty of Science, Chulalongkorn University for granting a teaching assistant fellowship during 1995-1997 and to the Graduate School for financial support as a part of this research work. In addition, I wishes to thank my committee, Professor Dr. Padet Sidisunthorn, Associate Professor Dr. Siri Varothai and Associate Professor Dr. Udom Kokpol for their comments and suggestions. I am also indebted to the Natural Products Research Unit, Department of Chemistry, Chulalongkorn University for help and support.

Special thanks must go to the selfless souls of persons listed below who provided me the help, friendship and understanding while preparing this thesis : Ms. Putaluk Khaiprapai, Ms. Siriphon Anyasimaphan and Mr. Sangchai Wattanasereekul.

Finally, I wish to thank my parents for their love and patience during the periods of time dedicated to this thesis.

Theerawut Wang-amnauporn

(Theerawut Wang-amnauporn)

CONTENTS

	Pages
Abstract in Thai	iv
Abstract in English	v
Acknowledgement	vi
List of Figures	ix
List of Schemes	x
List of Tables	xi
List of Abbreviations.....	xiii
Chapter	
I Introduction	
Background and Problematic Importance.....	1
Review of literature.....	3
Objective of this research.....	29
II Experimental	
Plant material.....	30
General procedure.....	30
Chemicals.....	30
Extraction for preliminary screening test.....	31
Extraction and initial fractionation.....	31
Synthesis.....	35
Bioassay experiment	
- Preparation of Greater Wax Moth	
<i>Galleria mellonella</i> Linn.	35
- Insect antifeedant test.....	36
III Results and Discussion	
The results of Extraction.....	37
General Extraction for Preliminary Insect Antifeedant Activity	
test.....	37
General Extraction.....	37

The Results of Insect Antifeedant Activity Screening Tests.....	41
Separation of Insect Antifeedant Activity from Active Crude	
Fractions.....	46
Separation of Crude Hexane.....	46
Separation of Fraction IB.....	48
Separation of Fraction IF.....	49
Structural Elucidation of Compound A.....	50
Structural Elucidation of Compound B.....	52
Insect antifeedant activity of related triterpenoids.....	56
Separation of Fraction IA.....	59
Structural Elucidation of Mixture C.....	60
Separation of Fraction IC.....	63
Structural Elucidation of Mixture D.....	64
Structural Elucidation of Mixture E.....	66
Separation of Fraction ID.....	69
Structural Elucidation of Compound F.....	71
Separation of Fraction IE.....	74
Separation of Crude Dichloromethane.....	75
Separation of Fraction IIID.....	77
Structural Elucidation of Compound G.....	79
Separation of Fraction IIIC.....	81
Separation of Fraction IIIE.....	82
Structural Elucidation of Mixture H.....	84
Structural Elucidation of Mixture I.....	85
Separation of Fraction IIIA.....	86
Separation of Fraction IIIB.....	87
The Separation of Crude Ethyl Acetate.....	87
Structural Elucidation of Compound J.....	89
IV Conclusion.....	93
REFERENCES.....	98
APPENDICES.....	101
VITA.....	112

List of Figures

Figures	Pages
1.1 Percentage of damage caused by all pests from FAO's statistical data.....	1
3.1 The insect antifeedant activity of crude extracts by the first extraction procedure.....	45
3.2 The insect antifeedant activity of crude extracts by the second extraction procedure.....	45
3.3 The insect antifeedant activity of each fraction separated from crude hexane...	47
3.4 Insect antifeedant activity of Compound A (acetyl aleuritolic acid).....	55
3.5 The insect antifeedant activity of each fraction separated from crude dichloromethane.....	77

List of Schemes

Schemes	Pages
2.1 Extraction procedure for the roots of <i>Trigonostemon reidioides</i> Craib.....	31
2.2 Extraction and fractionation by the first procedure.....	33
2.3 Extraction and initial fractionation by the second procedure.....	34
3.1 The result of extraction for preliminary insect antifeedant activity screening test.....	37
3.2 The results of extraction by method I.....	39
3.3 The results of extraction by method II.....	40
3.4 The preliminary insect antifeedant activity of the roots of <i>Trigonostemon reidioides</i> Craib.....	41
3.5 The insect antifeedant activity following the first extraction procedure.....	43
3.6 The insect antifeedant activity following the second extraction procedure....	44



 สถาบันวิทยบริการ
 จุฬาลงกรณ์มหาวิทยาลัย

List of Tables

Tables	Pages
1.1 The chemical constituents derived from <i>Trigonostemon reidioides</i> Craib.....	28
3.1 The results of extraction by method I.....	38
3.2 The results of extraction by method II.....	38
3.3 The results of insect antifeedant activity of crude extracts from the roots of <i>Trigonostemon reidioides</i> Craib. following the first extraction procedure.....	42
3.4 The results of insect antifeedant activity of crude extracts from the roots of <i>Trigonostemon reidioides</i> Craib. following the second extraction procedure.....	42
3.5 The results of separation of crude hexane by quick column chromatography technique.....	46
3.6 The insect antifeedant activity of each fraction separated from crude hexane.....	47
3.7 The results of the separation of Fraction IB.....	48
3.8 The results of the separation of Fraction IF.....	49
3.9 The IR absorption band assignments of Compound A.....	50
3.10 The ¹³ C-NMR chemical shift assignments of Compound A.....	51
3.11 The IR absorption band assignments of Compound B.....	52
3.12 The ¹³ C-NMR chemical shift assignments of Compound B.....	53
3.13 The results of insect antifeedant activity of Compounds A and B.....	54
3.14 The results of insect antifeedant activity of Compound A (acetyl aleuritolic acid).....	55
3.15 The results of insect antifeedant activity of Compounds A1 and A2.....	57
3.16 The results of insect antifeedant activity of triterpenoids.....	59
3.17 The results of the separation of Fraction IA.....	60
3.18 The IR absorption band assignments of Mixture C.....	61
3.19 The ¹³ C-NMR chemical shift assignments of Mixture C.....	62
3.20 The results of insect antifeedant activity of Mixture C.....	63
3.21 The results of the separation of Fraction IC.....	64
3.22 The IR absorption band assignments of Mixture D.....	65

3.23 The IR absorption band assignments of Mixture E.....	66
3.24 The ^{13}C -NMR chemical shift assignments of Mixture E.....	67
3.25 The composition of steroids in Mixture E.....	68
3.26 The results of insect antifeedant activity of Mixtures D and E.....	69
3.27 The results of the separation of Fraction ID.....	70
3.28 The IR absorption band assignments of Compound F.....	72
3.29 The ^{13}C -NMR chemical shift assignments of Compound F.....	73
3.30 The result of insect antifeedant activity of Compound F.....	74
3.31 The results of the separation of Fraction IE.....	75
3.32 The results of the separation of crude dichloromethane by quick column chromatography technique.....	76
3.33 The insect antifeedant activity of each fraction from crude dichloromethane.....	76
3.34 The results of the separation of Fraction IIID.....	78
3.35 The IR absorption band assignments of Compound G.....	79
3.36 The ^{13}C -NMR chemical shift assignments of Compound G.....	80
3.37 The result of insect antifeedant activity of Compound G.....	81
3.38 The results of the separation of Fraction IIIC.....	82
3.39 The results of the separation of Fraction IIIE.....	83
3.40 The IR absorption band assignments of Mixture H.....	84
3.41 The IR absorption band assignments of Mixture I.....	85
3.42 The results of insect antifeedant activity of Mixtures H and I.....	86
3.43 The results of the separation of Fraction IIIA.....	86
3.44 The results of the separation of Fraction IIIB.....	87
3.45 The results of the separation of crude ethyl acetate.....	88
3.46 The IR absorption band assignments of Compound J.....	89
3.47 The ^{13}C -NMR chemical shift assignments of Compound J.....	92

List of Abbreviations

br	broad
°C	degree celcius
cm ⁻¹	unit of wavelength
d	doublet (NMR)
DMSO	dimethylsulfoxide
g	gram (s)
GLC	gas liquid chromatography
J	coupling constant
kg	kilogram (s)
wt	weight
NMR	nuclear magnetic resonance
IR	infrared
L	liter (s)
m	multiplet (NMR)
mg	milligram (s)
mL	milliliter (s)
m.p.	melting point
ppm	part per million
s	singlet (NMR)
t	triplet (NMR)
δ	chemical shift
MeOH	methanol
EtOAc	ethyl acetate
R _f	retardation factor