UNIVERSITI PUTRA MALAYSIA

1. THE SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME ESTRAGOLE ANALOGUES TOWARDS OIL PALM POLLINATING WEEVILS ELAEIDOBIIUS KAMERUNICUS FAUST

2. ALKALOIDS CONSTITUENTS OF BREYNIA CORONATA (EUPHORBIACEAE)

HAZIMAH BTE ABU HASSAN

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2. ALKALOID CONSTITUENTS OF BREYNIA CORONATA (EUPHORBIACEAE)

By

HAZIMAH BTE ABU HASSAN

Thesis Submitted in Fulfilment of the Requirements for the Degree of Master of Science in the Faculty of Science and Environmental Studies
Universiti Pertanian Malaysia

August, 1990
DEDICATED TO KAK LONG AND AYIB
PREFACE

Plants and insects have evolved together over hundreds of million years that there exist between them, the most intricate interactions and interdependence. Plants are also very rich in chemicals which are apparently not directly connected with the normal metabolic process of photosynthesis, respiration and growth. This thesis consists of two studies: the first involves the ‘Synthesis and Biological Activity of Some Estragole Analogues Towards Oil Palm Pollinating Weevils Elaeidobius kamerunicus Faust’ and the second study involves the ‘Extraction of the Alkaloid Constituents of Breynia coronata (Euphorbiaceae).’

I would like to take this opportunity to express my sincere gratitude and appreciation to my supervisor, Assoc. Prof. Dr. Md. Nordin Hj. Lajis for his patience, support, guidance, suggestions, advice and discussion throughout this research work.

I am also grateful to my co-supervisor Dr. Mohd. Aspollah Hj. Sukari for his help and support, Dr. Abd. Rahman Manas for discussions and suggestions in some of the synthetic work, En. Atan Mohd. Shariff for discussions and help in obtaining some of the NMR spectra and Dr. Mawardi Rahmani and Dr. Sidek Silong for their help, directly or indirectly.
I am also indebted to Puan Nor Akma Ibrahim (Dept. of Math. UPM) for her time and patience during the discussion of the statistical analyses of the data, and Assoc. Prof. Dr. Mohd. Yussof Hussein (Dept. of Plant Protection, UPM) for some suggestions and permission to use his flowmeter for the bioassay tests.

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Last but not least, I am very grateful to my family especially to my parents, my husband Abd. Ghani Abdullah and my two children, Nur Suriyana and Ariff Ehsan for their love, support, sacrifices and understanding.

Finally I wish to thank Universiti Pertanian Malaysia for financial support through Graduate Assistant Programme.
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- Female Inflorescences of *E. guineensis* Jacq.
- *Breynia coronata* (Euphorbiaceae)
LIST OF ABBREVIATIONS

q  quartet
m  multiplet
d  doublet
t  triplet
h  heptet
s  singlet
b  broad
C  carbon
H  hydrogen
M  molar
Hz  Hertz
hr  hour
Lit  literature
min  minute
ppm  parts per million
mol  mole
no.  number
tlc  thin layer chromatography
b.p.  boiling point
m.p.  melting point
i.d.  internal diameter
mmol  millimole
THF  tetrahydrofuran
NMR  nuclear magnetic resonance
TMS  tetramethylsilane
BMS  borane methyl sulphide
IR  infra red
HPLC  high performance liquid chromatography
PORIM  Palm Oil Research Institute of Malaysia
MCPBA  m-chloroperbenzoic acid
PCC  pyridinium chlorochromate
EtOH  ethanol
Et2O  diethyl ether
CHCl3  chloroform
KOH  potassium hydroxide
CH2Cl2  dichloromethane
SOCl2  thionyl chloride
n-BuLi  n-butyl lithium
NaHCO3  sodium hydrogen carbonate
NaBH4  sodium borohydride
HCl  hydrochloric acid
NaOH  sodium hydroxide
MgSO4  magnesium sulfate
MeOH  methanol
CDCl3  deuterated chloroform
CrO3  chromium trioxide
Si  silica
Abstract of thesis submitted to the Senate of Universiti Pertanian Malaysia in fulfilment of the requirements for the degree of Master of Science.

**STUDY NO. 1**

THE SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME ESTRAGOLE ANALOGUES TOWARDS OIL PALM POLLINATING WEEVILS ELAEIDOBIUS KAMERUNICUS FAUST

**STUDY NO. 2**

ALKALOID CONSTITUENTS OF BREYNIA CORONATA (EUPHORBIAEAE)

By

HAZIMAH BTE ABU HASSAN

August, 1990

Supervisor: Assoc. Prof. Md. Nordin Hj. Lajis, Ph. D.

Faculty: Faculty of Science and Environmental Studies.

**STUDY NO. 1**

Estragole is an attractant for the weevils Elaeidobius kamerunicus Faust. The weevils were recently identified as an efficient pollinator of the oil palm, Elaeis guineensis Jacq.

Four organic compounds, analogous to estragole, namely: 4-ethoxyallylbenzene, 4-isopropoxyallylbenzene, 3-(4-methoxy phenyl)methyloxirane and (4-methoxyphenyl)acetaldehyde were prepared in the laboratory.
Bioassay using a Y-tube olfactometer was carried out for each analogue in order to test its activity as an attractant for the weevils *E. kamerunicus*. Attractancy tests against water indicated that none of the analogues were active except for 4-ethoxyallylbenzene.

However an intriguing result was observed when the activity of the compounds was tested against estragole. 3-(4-Methoxyphenyl)methyloxirane showed stronger activity when tested against estragole whereas all others were less attractive. It was thought that synergism was involved, however further tests carried out did not seem to indicate this was so.

The overall experiments showed that a slight change in the structure of the estragole caused some reduction in activity. The results also indicated that estragole is still the best attractant for the weevils *Elaeidobius kamerunicus* Faust.

**STUDY NO. 2**

Securinine, a yellow needle-shaped crystal, was successfully isolated from the leaves of *Breynia coronata* (Euphorbiaceae). A substantial amount was obtained and it was
easily purified by recrystallization from petroleum ether (b.p. 68-80°C). The structure elucidation was obtained from spectroscopic data and comparison with the literature.

The other two minor components were isolated and purified from gel chromatography. However, their structures could not be unambiguously determined due to their instability. Nevertheless, some of the spectroscopic analyses carried out indicated that they were also alkaloids of the securinine-type.
Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia bagi memenuhi syarat untuk mendapatkan Ijazah Master Sains.

KAJIAN PERTAMA

SINTESIS DAN KEAKTIFAN BIOLOGI BEBERAPA TERBITAN ESTRAGOLE TERHADAP KUMBANG PENDEBUNGA KELAPA SAWIT ELAEIDOBUIX KAMERUNICUS FAUST

KAJIAN KEDUA

KANDUNGAN ALKALOID BREYNIA CORONATA (EUPHORBIACEAE)

Oleh

HAZIMAH BTE ABU HASSAN

Ogos, 1990

Penyelia: Prof. Madya Md. Nordin Hj. Lajis, Ph. D.
Fakulti: Fakulti Sains dan Pengajian Alam Sekitar.

KAJIAN PERTAMA

Estragole adalah bahan penarik bagi kumbang Elaeidobius kamerunicus Faust. Kumbang ini telah dikenalpasti sebagai agen pendebungan yang berkesan bagi tanaman kelapa sawit dari spesies Elaeis guineensis Jacq.

Empat sebatian organik yang mempunyai struktur menyerupai estragole telah disediakan di makmal, iaitu 4-etoksialil-benzena, 4-isopropoksialilbenzena, 3-(4-metoksifenil)metil-oksirana dan (4-metoksifenil)asetaldehid.
Kajian biocerakan telah dijalankan ke atas sebatian-sebatian ini dengan menggunakan alat olfaktometer tiub-Y untuk mengkaji keaktifan biologinya sebagai penarik terhadap kumbang *E. kamerunicus*. Kajian perbandingan dengan air menunjukkan analog yang lain tidak aktif kecuali 4-etoksi alibenza.

Keputusan yang agak mengelirukan telah didapat apabila ujian perbandingan bagi semua sebatian ini dilakukan terhadap estragole. Walaupun kesemua sebatian termasuk 4-etoksi alibenza menunjukkan keaktifan penarikan yang lebih rendah, tetapi terbitan epoksida daripada estragole menunjukkan keaktifan yang lebih. Kajian lanjut telah dijalankan untuk memastikan kesan sinergism berlaku. Keputusan yang diperolehi bagaimanapun menunjukkan hipotesis ini tidak benar.

Keputusan keseluruhannya menunjukkan sedikit perubahan yang dilakukan terhadap estragole, telah mengurangkan keaktifannya sebagai penarik. Keputusan juga menunjukkan yang estragole tetap merupakan penarik yang baik untuk kumbang *Elaeodius kamerunicus* Faust.
KAJIAN KEDUA

Sekurinina, hablur kuning yang berbentuk jejarum, telah berjaya diasingkan dari daun *Breynia coronata* (Euphorbiaceae). Hasil yang diperolehi agak menggalakkan dan ianya mudah ditulenkan dengan kaedah penghabluran semula, menggunakan petroleum eter (s.d. 60-80°C). Penentuan strukturnya diperolehi daripada data spektroskopi dan membandingkannya dengan laporan literatur.

Dua komponen kecilnya diasingkan dengan menggunakan kaedah kromatografi gel Si. Strukturnya tidak dapat dipastikan kerana sebatian ini tidak stabil. Beberapa analisis spektroskopi berjaya dijalankan dan dari data yang diperolehi, menunjukkan kedua-dua komponen ini jenis alkaloid yang mempunyai rangka seperti sekurinina.
STUDY NO. 1

THE SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME ESTRAGOLE ANALOGUES TOWARDS OIL PALM POLLINATING WEEVILS ELAEIDOBUS KAMERUNICUS FAUST
CHAPTER 1
INTRODUCTION

_Elaeis guineensis_ - History, Products and Biology

Long before its introduction to Malaysia, the oil palm, _Elaeis guineensis_ Jacq. was abundantly found in the tropical Africa under natural conditions. The oil palm first entered this country through Singapore Botanical Gardens, but its first commercial planting began in 1917 at Tennamaran Estate in Kuala Selangor (Mohamad, 1966).

Malaysia exported 4.6 million tonnes of palm oil products in 1986, earning 3.5 billion ringgit of foreign exchange in the process. This placed the commodity as the third important foreign exchange earner after petrol and timber products. It has certainly replaced rubber and tin as the country's outstanding export (Lim, 1987).

Palm oil is used in the manufacture of soap, candles and edible products such as cocoa and milk (in the form of cocoa butter equivalents and the cocoa butter substitutes).
In addition to being a valuable shortening (frying oil), it is also a source of valuable ingredient in the making of ice-cream, confections and condiments.

Palm oil is also used extensively in the plating process, and to a small extent in the manufacture of heavy grease and lubricants. Its use as a fuel for internal combustion engines is still at the experimental stage.

Elaeis, Jacq. is a small genus of the family Palmae. They are found naturally in tropical Africa, where Elaeis guineensis Jacq. has a wide distribution. Elaeis melanococca Gaertn., occurs widely in tropical America. The genus is closely allied to Cocos (Burkhill, 1966).

Elaeis guineensis Jacq. is a large palm having a solitary columnar stem with a short internode. It is unarmed except for short spines on the leaf which give the palm its characteristic appearance (Hartley, 1967).

The palm is normally monoecious with male or female, but sometimes hermaphroditic, with inflorescences developing in the axils of the leaves. The fruit is a drupe which is borne on a large compact bunch. Being a monoecious plant, in which the male and female flowers occur separately, the palm tree
has distinct male and female inflorescences and in this case on the same plant.

The female inflorescence reaches a length of 30 cm or more before ripening. The spikelets are thick and fleshy and developed in the axils of a spinous bract. The flowers are arranged spirally around the spikelets with a sharp spine at the end of each spikelet. The inflorescence thus contains several thousands of flowers.

The male inflorescence is borne on a longer peduncle and contains long finger-like cylindrical spikelets, which is not spiney. The spikelet has bracts and terminal projections but the size are much reduced. The spikelets are between 10-20 cm in length (Hartley, 1967).

Literature Review

Pollination in *Elaeis guineensis*

The process of pollination involves the transfer of pollen from the male part of the plant to the receptive stigma of the female. How this occur in the oil palm plantations has been a matter of debates for sometimes (Kevan, 1983).

Oil palm pollination was believed to be anemophilous (wind-associated) rather than entomophilous (Jagoe, 1934; Gray
and Bevan, 1966; Wood, 1968; Hardon, 1973; Turner and Gillbanks, 1974). The belief stemmed from the supposedly lack of effective insect pollinators and this seemed to be substantiated by the high atmospheric pollen densities observed over considerable distances from the male inflorescences (Hardon and Turner, 1967). Hartley (1967) stated that the lack of pollens is a major cause in bunch failure and Hardon and Turner (1967) also observed that the production of the fruit bunch was greatly reduced during rainy seasons.

These observations led some planters in Cameroon, to suspect the involvement of other pollen dispersal agents, in view of the pollination in that country despite the wet seasons at which time rain occurs frequently.

Based on his work in Cameroon, Syed (1978, 1979, 1981a) has shown conclusively that insects are vital to efficient pollen transfer in oil palm plantations. The weevils *Elaeidobius kamerunicus* Faust was found to be one of the efficient oil palm (*Elaeis guineensis*) pollinators and with this discovery, Syed had disputed the concept of wind as a major pollinating agent and the role of insects was finally acknowledged.
Elaeidobius kamerunicus Faust

In West Africa, the natural habitat of oil palm, there are several pollinating insects (de Chenon, 1982) among which Elaeidobius, a species of small weevils are the most abundant.

Elaeidobius kamerunicus, the most important oil palm pollinator in Cameroon belongs to the sub-family of Derelominae of the family Circulionidae (Syed, 1979). The distributions and host of the genus Elaeidobius are remarkably restricted to Elaeis in West Africa. This is a good initial indication of the specialized nature of the sub-family.

The members of Derelominae in Cameroon include Elaeidobius kamerunicus, E. plagiatus, E. singularis, E. bilineatus, E. subvittatus and rarely E. spatulifer. They visit both male and female inflorescences of the oil palm, but Elaeidobius kamerunicus is the most abundant and efficient.

The females E. kamerunicus laid their eggs in the spent and pollen shedding male flowers, which were borne on the spikelets of the male inflorescence. When the eggs hatched the larvae fed on the drying, spent male flowers followed by pupating stage and later emerged as adults. This process takes about 10-13 days depending on the sex. Other species take either longer or shorter period to develop.