Control of African Striga Species by Natural Products From Native Plants.

Joseph Kipronoh Rugutt

Louisiana State University and Agricultural & Mechanical College

Follow this and additional works at: https://repository.lsu.edu/gradschool_disstheses

Recommended Citation
https://repository.lsu.edu/gradschool_disstheses/6371

This Dissertation is brought to you for free and open access by the Graduate School at LSU Scholarly Repository. It has been accepted for inclusion in LSU Historical Dissertations and Theses by an authorized administrator of LSU Scholarly Repository. For more information, please contact gradetd@lsu.edu.
INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6” x 9” black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI
A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
CONTROL OF AFRICAN STRIGA SPECIES BY NATURAL PRODUCTS FROM NATIVE PLANTS

A Dissertation
Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College
In the Partial fulfillment of the requirements for the degree of Doctor of Philosophy
in
The Department of Chemistry

by
Joseph Kipronoh Rugutt
BS., Moi University, Eldoret, Kenya, 1989
December, 1996
The Lord is good to all:
and his tender mercies are over all his works.

PSALMS 145:9
ACKNOWLEDGMENTS

In order to successfully accomplish research of this nature, there are a number of people who make invaluable contribution, both large and small. It is with great pleasure that I faithfully thank them.

This research has been accomplished under the direction of Dr. Nikolaus H. Fischer, to whom I would like to express my utmost appreciation for his endless moral as well as material support. Specifically, his endless academic support, guidance and encouragement has been a springboard of joy throughout the realization of this work. Dr. Fischer and Mrs. Helga Fischer are very pleasant, responsible and nice people to work with. You can always count on them in any situation. My association with the Fischers has meant more than words can describe. In short, their support of me and my family has been unfailing. I consider them second to none and rank them as World’s heroes. Also, I wish to sincerely acknowledge the remarkable assistance provided by Prof. Day and Mrs. Day. They constantly provided great personal encouragement and ascertained that everything kept going smoothly. Their long time friendship has been and will continue to be a never-ending source of pride and sustenance. I am also grateful to my wife Janny and my daughter Elizabeth who spent many lonely nights waiting for me to complete this research. Finally, I would like to thank Dr. Frank Fronczek for the determination of all the crystal structures; to Marcus Nauman for his invaluable help on the use of the NMR instrumentation; to Mr. Jeff Corkern for determination of GC/MS; Ms. Juliette Navratilova and Mr. Rostem Irani for isolation of pure compounds; Messrs. Charles Cantrell and Steven Robbs for their useful suggestions on structure elucidation; Dr. Berner and his technical staff for doing all bioassays; Mr. John Rugutt for statistical analyses; Dr. José Castañeda-Acosta for his help in the preparation of this manuscript; the committee members (Profs: Fischer, Cartledge, Watkins, Bhacca, Urbatsch, Moore and Berner) and the Rockefeller Foundation for financial support through African dissertation internship award.
# TABLE OF CONTENTS

ACKNOWLEDGMENTS ........................................................................................................ iii

LIST OF TABLES ................................................................................................................ vii

LIST OF FIGURES ............................................................................................................. ix

LIST OF SCHEMES .............................................................................................................. xii

ABSTRACT ........................................................................................................................... xiii

CHAPTER

I. INTRODUCTION ................................................................................................................ 1
   I.1. PEST MANAGEMENT ........................................................................................ 2
   I.2. PARASITIC PLANTS ......................................................................................... 2
   I.3. BIOLOGY OF STRIGA ..................................................................................... 3
   I.4. DISTRIBUTION OF STRIGA ........................................................................... 5
   I.5. THE STRIGA PROBLEM ................................................................................. 6
   I.6. PRACTICES TO CONTROL STRIGA SPECIES ............................................. 7
      I.6.1. Hand or Hoe Weeding ............................................................................. 8
      I.6.2. Land Preparation ..................................................................................... 8
      I.6.3. Rotation of Land into Fallow ................................................................. 9
      I.6.4. Breeding for Resistance/Tolerance ....................................................... 9
      I.6.5. Fertilizers ............................................................................................... 10
      I.6.6. Herbicides ............................................................................................. 10
      I.6.7. False-host or "Trap" Crops .................................................................... 10
      I.6.8. Germination Stimulants ...................................................................... 11
   I.7. QUANTITATIVE STRUCTURE-ACTIVITY (QSAR) ........................................... 16
      I.7.1. Basic Concepts of QSAR ...................................................................... 17
      I.7.2. Bioassays ............................................................................................... 18
   I.8. THE RESEARCH OBJECTIVES ......................................................................... 19

II. GC/MS EVALUATION OF COMPOUNDS IN DRY AND CONDITIONED STRIGA SEEDS .................................................... 24
    II.1. INTRODUCTION ......................................................................................... 25
    II.2. MATERIALS AND METHODS ..................................................................... 26
       II.2.1. Seed Collection .................................................................................... 26
       II.2.2. Seed Surface Disinfection and Conditioning ...................................... 26
       II.2.3. Preparation of Extracts of Striga Seeds .............................................. 27
       II.2.4. GC/MS Analysis ................................................................................ 27
    II.3. RESULTS .................................................................................................... 28
    II.4. DISCUSSION .............................................................................................. 29
    II.5. CONCLUSIONS .......................................................................................... 36

III. ACTIVITY OF EXTRACTS FROM NONHOST LEGUMES ON THE GERMINATION OF STRIGA HERMONTICA SEEDS ........................................................................ 42
    III.1. INTRODUCTION ....................................................................................... 43
    III.2. MATERIALS AND METHODS .................................................................... 44
       III.2.1. Collection of Plant Materials .............................................................. 44
       III.2.2. Preparation of Solutions of GR 24 (8) and GR 7 (9) ....................... 45
       III.2.3. Dichloromethane Extraction .............................................................. 45
       III.2.4. Water Extraction .............................................................................. 45
       III.2.5. Isolation of Sterols from Leaves ....................................................... 46
III.2.6. Thin-layer Chromatography (TLC) ................................................. 46
III.2.7. Bioassays .................................................................................. 47
   III.2.7.1. Crude Extracts ................................................................... 47
   III.2.7.2. Sterols ................................................................................ 48
III.2.8. Statistical Analysis .................................................................... 49
III.3. RESULTS ..................................................................................... 49
III.4. DISCUSSION ............................................................................. 51
III.5. CONCLUSIONS .......................................................................... 54

IV. IN VITRO GERMINATION OF STRIGA HERMONTICA AND
   S. ASPERA SEEDS BY 1-AMINOCYCLOPROPANE-1-ACID (ACC) .......... 72
IV.1. INTRODUCTION ......................................................................... 73
IV.2. MATERIALS AND METHODS ...................................................... 75
   IV.2.1. General ................................................................................ 75
   IV.2.2. Viability Test ......................................................................... 75
   IV.2.3. Preparation of ACC (16) and GR 24 (8) ................................ 75
IV.3. RESULTS ...................................................................................... 75
IV.4. DISCUSSION ................................................................................ 76
   IV.4.1. Mechanisms of Formation of Ethylene from ACC (16) ......... 77
   IV.4.2. Hypochlorite Chemistry ......................................................... 78
   IV.4.3. Possible Basic Mechanisms of Action of Ethylene on
           Germination of Striga Species Seeds ........................................ 80
IV.5. CONCLUSION ............................................................................. 84

V. STIMULATION OF STRIGA HERMONTICA GERMINATION
   BY 11βH,13-DIHYDROPARTHENOLIDE (DHP) .................................... 93
V.1. INTRODUCTION ......................................................................... 94
V.2. MATERIALS AND METHODS ...................................................... 95
   V.2.1. General ................................................................................ 95
   V.2.2. Seed Conditioning ................................................................. 95
   V.2.3. Measurement of Germination ............................................... 96
   V.2.4. Molecular Modeling ............................................................ 97
V.3. RESULTS ...................................................................................... 97
   V.3.1. Germination Data ................................................................. 97
   V.3.2. Molecular Mechanics Calculations ...................................... 98
V.4. DISCUSSION ................................................................................ 98
   V.4.1. Conformational Analyses ...................................................... 100
   V.4.2. The Stimulant-Receptor Interaction Model ............................ 101
   V.4.3. Solvents ............................................................................ 103
   V.4.4. Plausible Mechanism of Stimulation of Germination of
           Striga hermonthica Seeds by DHP (11) .................................. 104
V.5. CONCLUSIONS ........................................................................... 105

VI. ISOLATION OF DIHYDROELEPHANTOPIN, ISODEOXYELEPHANTOPIN AND GRINDELIC ACID FROM THE FAMILY
   ASTERACEAE ................................................................................. 110
VI.1. INTRODUCTION ....................................................................... 111
   VI.1.1. Germacrane ........................................................................ 111
   VI.1.2. Elephantopus tomentosus L ................................................ 112
   VI.1.3. Elephantopus carolinianus Willd ......................................... 112
   VI.1.4. Grindelia maritima L .......................................................... 112
VI.2. MATERIALS AND METHODS .................................................. 113
   VI.2.1. Isolation of Dihydroelephantopin (10) ............................... 113

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
VI.2.2. Isolation of Isodeoxyelephantopin (12) ........................................... 113
VI.2.3. Isolation of Grindelic Acid (14) .................................................... 113
VI.3. RESULTS AND DISCUSSION .............................................................. 114

VII. RESPONSE OF TWO DIFFERENT ISOLATES OF STRIGA
HERMONTICA SEEDS TO VARYING CONCENTRATIONS OF
STRIGOL ANALOG GR 7 ........................................................................ 127
VII.1. INTRODUCTION ........................................................................ 128
VII.2. RESULTS .......................................................................................... 129
VII.2.1. Effect of Several Concentrations of GR 7 (9) on the
Induction of Germination of Conditioned Striga
hermonthica Seeds ............................................................................. 129
VII.3. DISCUSSION ................................................................................... 129
VII.3.1. Selection Criteria for Germination Stimulants ................. 129
VII.3.2. Factors which affect Germinability of Striga
hermonthica Seeds .............................................................................. 130
VII.4. CONCLUSION .................................................................................. 132

VIII. CADINANES ISOLATED FROM HETEROTHECA SUBAXILLARIS
LAM ........................................................................................................ 135
VIII.1. INTRODUCTION ........................................................................ 136
VIII.2. EXPERIMENTAL ......................................................................... 137
VIII.2.1. Plant Materials ..................................................................... 137
VIII.2.2. Extraction and Isolation .......................................................... 137
VIII.2.3. NMR ......................................................................................... 138
VIII.3. RESULTS AND DISCUSSION ......................................................... 139
VIII.3.1. 13C NMR Spectral Assignments in 2-hydroxy-8α-
angeloyloxycalamenene (13) ................................................................. 139
VIII.3.2. 13C NMR Spectral Assignments in 2-hydroxy-8α-
hydroxycalamenene (42) .................................................................... 141

IX. COMPOUNDS ISOLATED FROM THE WEST AFRICAN HARRIS-
onia abyssinica OLIV. (SIMARUBACEAE).............................................. 161
IX. INTRODUCTION .............................................................................. 162
IX.1. Limonoids ......................................................................................... 162
IX.2. MATERIALS AND METHODS .......................................................... 165
IX.2.1. Structure Elucidation ................................................................. 165
IX.2.2. Isolation of Obacunone (45) ....................................................... 166
IX.2.3. Isolation of Pedonin (46), Harrisonin (47) and 12β-
acetoxyharrisonin (48) ................................................................. 167
IX.2.4. Isolation of (+)-ononitol (49) ...................................................... 167
IX.2.5. Bioassays ..................................................................................... 168
IX.3. RESULTS AND DISCUSSION .......................................................... 168
IX.3.1. Effects of (+)-ononitol (49) and Limonoids on Germina-
tion of Striga hermonthica Seeds ......................................................... 173

X. FUTURE RESEARCH ........................................................................... 201
REFERENCES ........................................................................................... 204
APPENDIX 1: Letter of Permission to use Published Materials ............... 225
VITA ............................................................................................................. 226

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
LIST OF TABLES

I.1. Striga species, their economic hosts and distribution ................................................................. 14

II.1. Relative area (%) of components in dichloromethane extracts of Striga seeds ................................................................. 37

III.1. Species and common names of legumes studied ................................................................................. 56

III.2. Percentage germination of conditioned Striga hermonthica seeds treated with aqueous solutions of strigol analogs, GR 24 (8) and GR 7 (9) ................................................................................. 57

III.3. Mean percentage germination of Striga hermonthica seeds treated with dichloromethane (DCM) or water extracts from leaves, stems and roots of legume cultivars ................................................................................. 58

III.4.1. Effect of dichloromethane (DCM) extracts from leaves of legume cultivars on germination percentages of Striga hermonthica seeds ................................................................. 59

III.4.2. Effect of dichloromethane (DCM) extracts from stems of legume cultivars on germination percentages of Striga hermonthica seeds ................................................................. 60

III.4.3. Effect of dichloromethane (DCM) extracts from roots of legume cultivars on germination percentages of Striga hermonthica seeds ................................................................. 61

III.5.1. Effect of aqueous extracts from leaves of legumes on germination percentages of Striga hermonthica seeds ................................................................................. 62

III.5.2. Effect of aqueous extracts from stems of legumes on germination percentages of Striga hermonthica seeds ................................................................................. 63

III.5.3. Effect of aqueous extracts from roots of legumes on germination percentages of Striga hermonthica seeds ................................................................................. 64

III.6. Source of variation in percentage germination of Striga hermonthica seeds as explained by the correlations (r²) ................................................................................. 65

III.7. Thin-layer chromatography (TLC) mobilities of compounds in dichloromethane (DCM) extracts ................................................................................. 66

III.8. Composition (%) of sterols isolated from leaves ................................................................................. 68

V.1. Calculated conformational energies (kcal/mol) of strigol (2), GR 24 (8) and DHP (11) ................................................................................. 108

VIII.1. 13C-H correlation of 2-hydroxy-8α-angeloyloxy calamene (13) (400 MHz, CDCl₃) ................................................................................. 145

VIII.2. 1H- and 13C NMR spectral data (8) for 2-hydroxy-8α-hydroxy-calamene (42) (400 MHz, CDCl₃) ................................................................................. 146
IX.1. $^{13}\text{C}-^1\text{H}$ correlation of obacunone (45) (400 MHz, CDCl$_3$).......................... 174
IX.2. Coordinates and equivalent isotropic thermal parameters................................. 177
IX.3.1. Selected bond lengths (Å)........................................................................... 178
IX.3.2. Selected bond angles (deg.)......................................................................... 178
IX.3.3. Selected torsion angles (deg.)....................................................................... 179
IX.4. Percentage germination of conditioned Striga hermonthica seeds
treated with aqueous solutions of obacunone (45), pedonin (46),
harrisonin (47) and (+)-ononitol (49)....................................................................... 180
LIST OF FIGURES

I.1. Compounds investigated for Striga seed germination ................................................. 21

II.1 A and B. Total ion chromatogram profiles of compounds detected in dichloromethane extracts of dry (A) and conditioned (B) Striga hermonthica seeds ................................................................. 39

II.2 A and B. Total ion chromatogram profiles of compounds detected in dichloromethane extracts of dry (A) and conditioned (B) Striga gesnerioides seeds ........................................................................................................ 40

II.3 A and B. Total ion chromatogram profiles of compounds detected in dichloromethane extracts of dry (A) and conditioned (B) Striga aspera seeds ........................................................................................................ 41

III.1. Effect of chondrillasterol (15) on germination of conditioned Striga hermonthica seeds occurring in Bida (A), Kano (B) and Abuja (C and D) locations in Nigeria ........................................................................ 69

III.2. Effect of chondrillasterol (15) on germination of conditioned Striga aspera seeds occurring in Kano (E) location in Nigeria ........................................................................................................... 71

IV.1. Effect of ACC (16) on germination of isolates of conditioned Striga hermonthica seeds occurring in Bida (A), Kano (B) and Abuja (C and D) locations in Nigeria ........................................................................ 90

IV.2. Effect of ACC (16) on germination of isolate of conditioned Striga aspera seeds collected in Kano location (E) in Nigeria ........................................................................................................... 92

V.1. Effect of dihydroelephantopin (DHP) (11) on germination of conditioned Striga hermonthica seeds ................................................................................................................................. 107

VI.1. Effect of dihydroelephantopin (10) and isodeoxyelephantopin (12) on germination of isolate of conditioned Striga hermonthica seeds occurring in Bida (A), Kano (B) and Abuja (C and D) locations in Nigeria ........................................................................................................... 119

VI.2. Effect of dihydroelephantopin (10) and isodeoxyelephantopin (12) on germination of isolate of conditioned Striga aspera seeds collected in Kano location (E) in Nigeria ........................................................................................................... 123

VI.3.1. Effect of grindelic acid (14) on germination of conditioned Striga hermonthica seeds occurring in Bida (A), Kano (B) and Abuja (C and D) locations in Nigeria ........................................................................................................... 124

VI.3.2. Effect of grindelic acid (14) on germination of isolate of conditioned Striga aspera seeds collected in Kano location (E) in Nigeria ........................................................................................................... 126

VII.1. Effect of GR 7 (9) on germination of conditioned Striga hermonthica seeds collected in Abuja (A and B) and Bida (C) locations in Nigeria ........................................................................................................... 133
VIII.1. DEPT 90, 135 and BB $^{13}$C NMR spectra of 2-hydroxy-8α-angeloyloxycalamenene (13) ................................................................. 147

VIII.2. 2D $^1$H NMR COSY spectrum of 2-hydroxy-8α-angeloyloxycalamenene (13) ................................................................................................. 148

VIII.3. 2D $^1$H NMR NOESY spectrum of 2-hydroxy-8α-angeloyloxycalamenene (13) ................................................................. 149

VIII.4.1. 2D $^{13}$C-$^1$H HETCOR spectrum of 2-hydroxy-8α-angeloyloxycalamenene (13) ........................................................................................ 150

VIII.4.2. Upfield region of HETCOR spectrum of 2-hydroxy-8α-angeloyloxycalamenene (13) .................................................................................. 151

VIII.5. Downfield region of COLOC spectrum of 2-hydroxy-8α-angeloyloxycalamenene (13) ................................................................. 152

VIII.6. 2D $^1$H NMR COSY spectrum of 2-Hydroxy-8α-hydroxycalamenene (42) ................................................................................................. 153

VIII.7. 2D $^1$H NMR NOESY spectrum of 2-Hydroxy-8α-hydroxycalamenene (42) ................................................................................................. 154

VIII.8. DEPT 90, 135 and BB $^{13}$C NMR of 2-Hydroxy-8α-hydroxycalamenene (42) ................................................................................................. 155

VIII.9.1. The 2D $^{13}$C-$^1$H HETCOR spectrum of 2-Hydroxy-8α-hydroxycalamenene (42) ................................................................................................. 156

VIII.9.2. Upfield region of HETCOR spectrum of 2-Hydroxy-8α-hydroxycalamenene (42) ................................................................................................. 157

VIII.9.3. Effect of 2-Hydroxy-8α-angeloyloxycalamenene (13) on germination of conditioned S. hermonthica seeds occurring in Bida (A), Kano (B) and Abuja (C and D) locations in Nigeria ........................................ 159

VIII.9.4. Effect of 2-Hydroxy-8α-angeloyloxycalamenene (13) on germination of conditioned S. aspera seeds occurring in Kano (E) location in Nigeria ........................................ 160

IX.1. DEPT 90, 135 and BB $^{13}$C NMR spectra of obacunone (45) ................................................................................................. 181

IX.2. 2D $^1$H NMR COSY of obacunone (45) ................................................................................................. 182

IX.3. 2D $^1$H NMR NOESY spectrum of obacunone (45) ................................................................................................. 183
IX.4.  2D $^{13}$C-$^1$H HETCOR spectrum of obacunone (45) .................................. 184
IX.5.  COLOC spectrum of obacunone (45) ................................................................. 185
IX.6.  $^1$H NMR spectrum of pedonin (46) ................................................................. 186
IX.7.  2D $^1$H NMR COSY spectrum of pedonin (46) .................................................. 187
IX.8.  2D $^1$H NMR NOESY spectrum of pedonin (46) .............................................. 188
IX.9.  $^{13}$C NMR spectrum of pedonin (46) ................................................................. 189
IX.10. DEPT 90, 135 and BB $^{13}$C NMR spectra of pedonin (46) .............................. 190
IX.11. 2D $^{13}$C-$^1$H HETCOR spectrum of pedonin (46) ........................................... 191
IX.12. 2D $^{13}$C-$^1$H COLOC spectrum of pedonin (46) ............................................... 192
IX.13.  $^1$H NMR spectrum of harrisonin (47) .............................................................. 193
IX.14.  Downfield region of COSY spectrum of harrisonin (47) ................................. 194
IX.15.  2D $^1$H NMR NOESY spectrum of harrisonin (47) ........................................... 195
IX.16.  $^1$H NMR spectrum of 12β-acetoxyharrisonin (48) ......................................... 196
IX.17.  2D $^1$H NMR COSY spectrum of 12β-acetoxyharrisonin (48) ......................... 197
IX.18.  2D $^1$H NMR NOESY spectrum of 12β-acetoxyharrisonin (48) ....................... 198
IX.19.  BB $^{13}$C NMR spectrum of 12β-acetoxyharrisonin (48) ................................. 199
IX.20.  DEPT 90, 135 and BB $^{13}$C NMR spectrum of 12β-acetoxyharrisonin (48) ........ 200
LIST OF SCHEMES

I.1. Life cycle of *Striga* ................................................................. 4

I.2. Berner *et al.* (1995) scheme of integrated *Striga*-control (modified to include chemical control) .................................................. 8

I.3. Oxidation of dihydrosorgoleone (3) to quinone (4) ............................ 15

I.4. Reduction of methylene blue (5) with hydroquinone (3) to yield the transparent reduced form (6) ......................................................... 15

I.5. Extraction and bioassays of germination stimulants and/or inhibitors 23

IV.1. Mechanism of Adams and Yang (1979) for the biosynthesis of ethylene ..................................................................................... 85

IV.2. Formation of ethylene by the chemical oxidation of 1-phenylcyclopropylamine (Hiyama *et al.*, 1975) ............................................. 86


IV.4. Proposed mechanism of Lizada and Yang (1979) for the oxidation of ACC with hypochlorite ............................................................. 88

IV.5. A possible mechanism of action of ethylene .................................. 89

V.1. Proposed mechanism for biosynthesis of ethylene from DHP (11) 109
ABSTRACT

Obligate root-parasitic flowering plants of the genus *Striga* (family Scrophulariaceae) cause considerable yield reductions of various crops in tropical and semi-tropical countries. Mature *Striga* species (witchweeds) plants produce copious quantities of minute seeds which remain dormant in the soil for many years until exudates from roots of various host plants induce germination. Economically effective means of control of *Striga* species are not yet available for small-scale farmers in developing countries. In the present study data is presented which indicate that the two most effective *Striga* control measures are crop rotation and suicidal germination.

For the first time we have analyzed the dichloromethane extracts of dry and conditioned *Striga* species seeds by means of gas chromatography coupled to mass spectrometry. Sixteen compounds were identified on the basis of their mass spectra and their retention indices. All *Striga* species extracts contained tetradecanoic acid, *cis,cis*-9,12-octadecadienoic acid, *cis*-9-octadecenoic acid and sitosterol. Also, 2,6-dimethoxy-p-benzoquinone (2,6-DMBOQ) and several long chain aldehydes and n-hydrocarbons were detected in some of the extracts. The nature of the chemical changes induced by seed conditioning are discussed.

Dichloromethane and water extracts of various parts of legume cultivars were tested for stimulation of germination of *Striga* species seeds as described in the experimental sections. Also, several pure compounds isolated were assayed. Dilution methods, which are very sensitive and better suited for quantifying germination stimulant activity, were employed in identification of high and low stimulant producing legume cultivars. New and interesting relationships between stimulatory activity and concentration emerged. Most pure compounds tested induced significant germination of isolates of *Striga* species seeds across a broad concentration range, from $10^{-3}$ to $10^{-20}$ M. The mechanism of *Striga* germination proposed may foster synthesis of more effective germination stimulants and/or inhibitors.
I.1. PEST MANAGEMENT. Producing enough food for an ever-growing population is the biggest problem facing the human race. Efficient use of diminishing farmland and maximization of crop returns is becoming ever more prevalent. The difficulty of this is not eased by the fact that, worldwide, a large proportion of crops are lost to insects, diseases, weeds, and parasitic plants. In the developing world where farmers and governments struggle to feed hungry mouths, the cost of the damage caused by these pests on agriculture is of utmost concern.

For many years, the main weapons against pests were chemical. From the early sprays of tar and copper sulfate to the sophisticated modern cocktails, chemical pest control has had much success. Traditionally, the agricultural community has relied extensively on synthetic pesticides such as organo-chlorines, -phosphates, and dinitrophenols (Ley et al., 1993 and references therein). Chemicals, however, undergo biological amplification in the food chain and can be harmful to wildlife, the environment, and humans. Furthermore, they are expensive, and farmers may have to use more and more because the pests develop resistance to chemicals. For these and other reasons, there is an urgent need to develop alternative and ecologically acceptable methods of controlling the pests.

I.2. PARASITIC PLANTS. Parasitic plant classification is based on several criteria. Holoparasites lack chlorophyll and depend entirely on the host for nutrients and minerals; examples include Cuscuta (Cuscutaceae) and Orobanche spp. (Orobanchaceae) (Calder and Eager, 1979). Hemiparasites have chlorophyll and thus can fix carbon dioxide but depend on the host for water and minerals; examples include Striga spp. (Scrophulariaceae) and mistletoes (Loranthaceae). Some parasites attack the shoot, others the root. Parasites that require attachment to the host to complete their life cycle are obligate parasites whereas facultative parasites can complete their life cycles without the host.
From an evolutionary standpoint, parasitic plants have lost many organs and they have also evolved a unique set of characteristics essential to their mode of life. The haustorium is the most important of these. Early anatomical evidence suggested that the haustorium of parasitic plants served only as a bridge, transporting sap from host to parasite. More recent studies show that the organ has a complex structure (Musselman, 1987). The haustorium is primarily involved not only with transport but also with the metabolism of nutrients received from the host. The parasite and its host contain different sugars and amino acids, and the haustorium cells may be responsible for converting nutrients from the host into a form more suitable to the parasite. The genesis of haustoria is still a subject of speculation. It is envisioned that the roots of the parasite penetrate those of the host by both mechanical and enzymatic action.

1.3. BIOLOGY OF STRIGA. The genus *Striga* (Scheme I.1), which belongs to the family Scrophulariaceae, is among the most agronomically important parasites particularly in Africa. All *Striga* species are obligate root parasites that strongly affect the food resources of Africa and Asia. Botanically, the genus is characterized by opposite leaves, irregular flowers with a corolla divided into a tube and spreading lobes, herbaceous habit, small seeds, and parasitism (Musselman, 1987). In the field, *Striga*, or witchweed as it is commonly known, looks like an ordinary green weed and has very attractive flowers. It earned its common name “witchweed” because although farmers realized that the parasite's appearance in a crop meant a large loss of yield, the farmers did not understand the weed's parasitic nature. As far as the farmer was concerned, the crop had been bewitched (Nour et al., 1986). In fact the word "*Striga*" in Latin means witch, and describes the condition of the host plant, which (when the parasite is still invisible in the soil) expresses apparently inexplicable disease symptoms.

*Striga*'s unique parasitic lifestyle and has allowed its adaptation to the humid and semi-arid Savannas and Sahel regions in the tropics. Parasitism is a biological attempt to gain something for nothing, and *Striga* is evolutionarily adapted to this mode of life.
Scheme I.1. Life cycle of *Striga*.
Surprisingly, the green photosynthetically active plants of Striga rely on the host for most of their carbohydrates. Research (Nour et al., 1986) has shown that mature Striga plants have low rates of photosynthesis, even though the structure of their chloroplast is normal. One reason is that the key enzyme of photosynthesis, ribulose bisphosphate carboxylase, is present only in small amounts. Thus the overall biosynthetic activity of Striga is low. At the metabolic level, Striga differs from its host in a number of ways. One notable difference is that mannitol, a sugar alcohol, is an end product of photosynthesis in Striga, but is absent from hosts such as sorghum and millet. This difference raises an attractive possibility that a substance targeted against the metabolism of mannitol might be an effective herbicide against photosynthesizing Striga spp.

I.4. DISTRIBUTION OF STRIGA. As a whole, the genus Striga comprises about 35 species in the Old World tropics. Striga spp. did not exist in the New Continent but were recently been introduced (Musselman, 1987). The diversification of the genus occurred first on the Old Continent (Table I.1). A secondary diversification center may be Australia. Considering diversity and endemcity, the main diversification center of the genus seems to be located in the West- and Central Africa. It is probable that the differentiation of Striga species arose after geographic separation of the two continents.

The majority of Striga species are of no agronomic importance, but those that parasitize tropical cereals and legumes can be extremely damaging. These economically important species include S. asiatica (L.) Kuntze, S. aspera (Willd.) Benth, S. hermonthica (Del.) Benth and S. gesnerioides (Willd.) Vatke. S. hermonthica, is without doubt one of the most important parasitic weeds of the world. It is the most damaging species in sub-Saharan Africa, affecting staple cereals such as maize, sorghum, millet, rice and sugarcane. It is worth noting that maize, sorghum and millet are among the world's most important food crop, and for the inhabitants of the sub-Saharan Africa, they are the main sources of protein and energy (Wendorf et al., 1992). With the possible exception of birds, S. hermonthica may be the single most important cause of yield loss.
reduction in sorghum and millet in the whole of Africa (Parker and Riches, 1993). It is estimated that *Striga* species threaten the lives of over 100 million people in Africa and infest two-thirds of the 73 million hectares devoted to cereal crop production in African savannas alone (Lagoke *et al.*, 1991). The greatest damage occurs in the savanna areas stretching from Cape Verde on the west coast through west, central, east and southern Africa. These zones also constitute the major food legume producing areas in most of the countries affected (Robson and Broad, 1989). For example, in Nigeria alone, the most populous country in Africa, *S. hermonthica* causes crop losses of 10 to 90 percent in maize and sorghum.

In 1956, the parasitic weed *S. asiatica* was found in the USA (Wunderlin *et al.*, 1979). This parasite has a wide geographic distribution and broad range of hosts including many important crops (Saldhanha and Nicolson, 1976). In January 1979, an infestation of *S. gesnerioides* was reported in central Florida. This species is parasitic on a number of broad-leaved plants in the family Leguminosae (Cowpea, Soybean, *Tephrosia pedicellata* Bak.), including Indigofera, and members of various other plant families, including Solanaceae, Convolvulaceae (*Ipomea*, e.g. Sweet potatoes; *Jacquemontia tamnifolia* (L.) Griseb. and *Merremia tridentata* L.), Euphorbiaceae (*Euphorbia*), Liliaceae (*Sansevieria*), Acanthaceae (*Lepidagathis*) (Wunderlin *et al.*, 1979) and also some Gramineae. This parasite causes leaf chlorosis, die-back and malformation of parasitized cowpea plants. Yield losses due to *S. gesnerioides* infestation range from 26 to 50% (Aggarwal, 1987).

### I.5. THE STRIGA PROBLEM.

*Striga* spp. thrive in areas of low soil fertility and low rainfall, causing problem for small-scale resource-poor farmers, who are often confined to these agronomically poor areas. Control of *Striga* is more difficult than the control of non-parasitic weeds (Ogborn, 1987). The *Striga* spp. life cycle (Scheme I.1) is closely coupled to their environment and to their host. *Striga* species produce large quantities of very tiny seeds which can remain dormant in the soil up to 14 years. The
seeds will only germinate on exposure to both favorable environmental conditions and to a germination stimulant, usually a host-root exudate. A single *Striga* plant can produce up to 500,000 seeds each as small as 5µg (200,000 seeds per gram). These seeds have complex dormancy and germinate at different times. Therefore they can parasitize crops over an extended period. As many as 50 *Striga* plants can parasitize a single host plant. The control problem of *Striga* is compounded by the fact the very tiny seeds are spread predominantly by activities of man (Bemer et al., 1995).

*Striga* species exhibit great genetic variability and form haustoria as a common feature. Through this special organ, the parasites attach themselves to the roots of the host plants and do considerable damage before they emerge above the ground. *Striga* damages food production in three ways. First, there is a direct loss in yield because of parasitism. The high-yielding hybrids of sorghum developed recently by Nour et al. (1986) are particularly vulnerable. Secondly, the small-scale African farmers lose by switching to low-yielding crops, such as millet, which are less susceptible but have lower yields. Thirdly, farmers abandon land as a result of heavy infestation. In some areas, whole villages move to escape the parasite infestations.

**I.6. PRACTICES TO CONTROL STRIGA SPECIES.** The widespread and severe problem of parasitism by *Striga* spp. in crop production differs from that caused by other weed species. *Striga* spp. damage their hosts through parasitism and induction of disease as opposed to weeds which cause damage by competition. *Striga* spp. seeds possess complex dormancy and not all the seeds conditioned for germination at the same time. This compounds the problem of devising a persistent and adequate control method. Among the measures employed to combat the parasite are hand-weeding, "trap" and "catch" cropping, use of nitrogen fertilizers, herbicides, resistant crop varieties, and germination stimulants (Musselman, 1980; Ayensu et al., 1984; Lagoke et al, 1991). The control measures vary in effectiveness, cost, and knowledge required by the farmer. None of these measures by itself seems to provide effective control. An integrated
package involving various methods will be the most effective approach to control. The article by Berner et al. (1995) gives a complete coverage of Striga research in Africa. Although our attention is focused on the use of germination stimulants in Striga spp. control (Scheme 1.2), an overview of Striga control measures implementable by small-scale African farmers is presented below.

\[\text{Striga-free Planting Material}\]
\[\text{Chemical Control}\]
\[\text{Biocontrol}\]
\[\text{Host Seed Treatments}\]
\[\text{Crop Rotation}\]
\[\text{Transplanting}\]
\[\text{Host-Plant Resistance}\]

Scheme 1.2. Berner et al. (1995) scheme of integrated Striga-control (modified to include chemical control).

1.6.1. **Hand or Hoe Weeding.** This is the most common practice used by small-scale farmers. It is worth noting that Striga does most of its damage to the host crop before they emerge. Hand pulling could be effective in controlling a light infestation and especially if done before or during the flowering of Striga (Pieterse, 1985). However, hand pulling is a very tedious, labor-intensive and expensive operation.

1.6.2. **Land Preparation.** This practice has been tested by the United Nations (UN) Food and Agriculture Organization (FAO) in West Africa. It was believed that if tillage could be avoided, Striga seeds would remain on the surface of the soil where conditions would eliminate their chances of attacking the crop (Parker and Reid, 1981). This method did not work well in most of the trials. Deep cultivation to bury the Striga seeds
has been used in some parts of Africa for quite some time. The effectiveness of this method is limited since the seeds remain viable and can resurface over time. Moreover, deep cultivation is not only tedious and expensive but may not be very practical to the African farmers especially considering their rudimentary farm machinery.

1.6.3. Rotation of Land into Fallow. This was once the traditional farming system of most of the affected areas. Land was allowed to return to bush fallow for 10 to 20 years during which time the Striga seed bank in the soil would become depleted and crops could be grown again. Where practiced, the rotation periods have declined such that there is little effect on the Striga seed population. This method is limited by increasing human population.

1.6.4. Breeding for Resistance/Tolerance. The development of resistant/tolerant crop cultivars is one reliable approach to controlling Striga. Much effort is taking place within crop breeding departments of national research programs and at the International Institute of Tropical Agriculture (Parker and Riches, 1993). Considerable progress has been made, but there are difficulties. Resistant or tolerant crop cultivars can have limitations such as low quality grain. Also, new Striga strains occur or may emerge that thwart resistance (Musselman, 1987). In effect, all resistant or tolerant crop varieties allow infection by the parasite to varying degrees and this provides sources of re-infestation. Furthermore, breeding for resistance to Striga requires effective screening techniques. The development of reliable methods for screening large numbers of host genotypes in the field has been effective through establishment of uniform levels of infestation for reliable and reproducible results (Kim, 1991).

1.6.5. Fertilizers. Striga spp. are most abundant on cereals and very damaging on soils of low fertility. There are many conflicting reports on the control of Striga through management of soil fertility (Pieterse and Pesch, 1983). The application of nitrogenous fertilizers may reduce infestation (Pesch et al., 1983), but these fertilizers remain unavailable in many parts of Africa and are often too expensive for many farmers.
1.6.6. **Herbicides.** Direct application of foliar herbicides after the emergence of parasitic weeds has been observed to give effective reduction of the parasites, but the cost and the need to take precautions to avoid health hazards make this practice difficult. It should be noted that the damage by *Striga* spp. is done before emergence.

1.6.7. **False-host or "Trap" Crops.** "Trap" crops are nonhosts which stimulate germination of the *Striga* seeds without being attacked by the seedlings. When seedlings do not find a suitable host they use up their endosperm and die within 3-7 days. Trap crops can be cultivated alone or they can be rotated with a susceptible host crop, thus eliminating the parasitic weed seeds that would otherwise germinate and attack the susceptible host crop. This control approach is a promising method and is economically advantageous for the farmer (Musselman, 1987). Since the false-host is a crop, it is economically viable for the farmer. Also, unlike many other inexpensive control methods, the false-host crop reduces the parasitic weed seed numbers in a field, thus reducing the probability of more severe future crop damage. However, little is known about false-host crops and the compounds responsible for stimulation (Lagoke *et al.*, 1991). More research is needed to identify the most effective plant species and cash and food crop cultivars that can serve as false-hosts. Also, false-hosts must be identified that address the problem of the diversity of *Striga* spp. in the same field.

Although control of *Striga* through use of non-host crops in rotation appears to be an attractive measure, several complicating factors hinder successful implementation. One of these is the diversity of *Striga* spp. that can be found in farmer's fields. A control practice for one may be ineffective for the other(s). Of particular complexity for control is the common admixture of *S. gesnerioides*, a parasite of cowpea (*Vigna unguiculata* (L.) Walp.), and *S. hermonthica*, a parasite of most cereal crops in Africa. Cowpea is a common and important food source particularly in West Africa, but can be severely damaged by *S. gesnerioides*. Because cowpea is not a host of *S. hermonthica* but can stimulate *S. hermonthica* seed germination, certain cowpea cultivars can be used in...
rotation with cereals to provide *S. hermonthica* control. However, few non-hosts have been identified to provide complementary control of *S. gesnerioides* on cowpea. Use of rotation crops such as soybean and forage legumes, which are nonhosts to either parasite, present a solution to this problem. Variability among cultivars of nonhosts in their ability to stimulate parasite seed germination can be utilized and the most effective cultivars /germplasm selected. Literature indicate that little work has been done on identifying efficacious cultivars of nonhosts for *S. hermonthica* and *S. gesnerioides* control (Ariga et al., 1993).

1.6.8. **Germination Stimulants.** In addition to the normal germination requirements common in many angiosperms, dormant *Striga* seeds germinate only in response to chemical signals emitted by the roots of suitable plants (Chang et al., 1986; Netzly et al., 1988; Worsham, 1988). In the absence of a stimulant-producing plant, ethylene (1), strigol (2) and strigol analogues can, in the immediate vicinity (4 mm) of the *Striga* spp. seeds, induce suicidal ("abortive") germination of *Striga* seeds in the soil (Eplee, 1975; 1984). This leads to a reduction in the *Striga* seed bank in the soil before a crop is planted. In the United States, *S. asiatica* is effectively eradicated using ethylene (1), a natural, ubiquitous compound, is injected into the soil (Eplee, 1992; Sand and Manley, 1990). When properly used, this method can be highly effective (Eplee, 1983). In developing countries, this method is of limited potential use because ethylene (1) is a gas under relatively high pressure and its use for large-scale control requires a high level of skill as well as transport and application equipment that are not available or are prohibitively expensive for the farmers in developing countries (Ogborn, 1987; Sand and Manley, 1990). Moreover, drier soil conditions (Bebawi and Eplee, 1986) reduces the efficacy of ethylene (1) on stimulation of *Striga* spp. seeds in Africa. For these reasons the search for other naturally derived inexpensive, and easily applied germination stimulants for control of *Striga* seems continues.
Root exudates of natural host plants such as cereals and legumes produce, in infinitesimal levels, a mixture of very active stimulatory compounds with one compound being predominant over the others (Worsham, 1987; Weerasuriya et al., 1993). From the natural host sorghum, host-specific signals, collectively called sorgoleones, were recently isolated (Chang et al., 1986; Netzly et al., 1988). The highly unstable sorgoleone 2-hydroxy-5-methoxy-3-[(8'Z, 11'Z)-8',11'14'-pentadecatriene]-p-hydroquinone (3) is rapidly oxidized to non-stimulatory 2-hydroxy-5-methoxy-3-[(8'Z, 11'Z)-8',11'14'-pentadecatriene]-p-quinone (4) (Scheme 1.3). Since the hydroquinone (3) appears to be the essential component of the exudate in the induction of Striga germination, methods have been designed to specifically localize hydroquinone production along the sorghum root surface (Chang, 1986 and Chang et al., 1986). Methylene blue (5), a commercially available heterocyclic pigment, can be reduced electrochemically ($E^0' = +20 \text{ mV at pH 7.6}$) (Clark, 1960) or with chemical reductants like sodium dithionite. The hydroquinone, structure (3), reacts stochiometrically with methylene blue (5) to give the visibly transparent $2e^-$ reduced form. In the presence of $O_2$, the reduced dye autoxidized back to the blue form (6) (Scheme 1.4) and structure 3 has been isolated intact. Therefore, in the presence of $O_2$, this pigment can be used to investigate the persistence of the production of structure 3. The oxidative lability of the crude exudate from sorghum has already been documented (Chang et al., 1986), and the t1/2 for biological activity of a $10^{-5} \text{ M}$ solution in $H_2O$ at room temperature range from 12 to 24 h. The rate of autoxidation of the pure hydroquinone (3) as quantified by UV and HPLC has been found to be at least two orders of magnitude faster than the exudate. In general, the root exudates of grasses have been shown many years ago to be very complex mixtures containing sugars, amino acids, organic acids, nucleotides, phenolics, and enzymes (Rovira, 1965). Specifically, hydroquinone (3) is a general feature of Striga's hosts. It is possible that such a metabolically expensive process could be defensive in nature. In that regard, related quinones have been shown to be metabolic toxins (Wong et al., 1985).
serving as phytoalexins (Bundenburg et al., 1949). The autoxidation of the hydroquinone (3) results in the generation of reactive and toxic oxygen intermediates. The bis-allylic methylenes of the side chain are also known to react with oxygen radicals. Related compounds containing both catechol (7) and olefinic (C=C) functionalities polymerize (Takada et al., 1988) and, in fact, formed the basis of the original lacquer industry. Such a polymerization may generate other structures of relevance to the rhizosphere or a polymerization may even function to stabilize the soil around the roots. Any of these mechanisms could provide general protection for the sorghum roots. A more appealing possibility would involve a specific role for these compounds. Azospirillum spp. and nitrogen fixing bacteria colonize many of the same species that Striga parasitizes (Dobereiner and Pedroza, 1987). These exudates could induce specific root colonization by reducing the oxygen tension around the host root (most of these bacteria can multiply and fix N\textsubscript{2} only at reduced O\textsubscript{2} pressures), serving as a specific reductant or terminal electron acceptor for bacterial respiration, or even as a specific bacterial chemoattractant (Egley and Dale, 1970). Any specific role that these compounds might play awaits further investigations.

Ethylene (1)

\[ \text{H} = \text{C} = \text{H} \]

Catechol (7)

\[ \text{C} - \text{OH} \]

\[ \text{C} - \text{OH} \]

(+)-strigol (2)

\[ \text{A} - \text{B} - \text{C} - \text{D} \]
Table 1.1. *Striga* species, their economic* hosts and distribution

<table>
<thead>
<tr>
<th><em>Striga</em> species</th>
<th>Host Crop</th>
<th>Countries</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. hermonthica</em></td>
<td>sorghum, millet, maize, upland rice, fonio, tef, sugarcane, finger millet, lowland rice</td>
<td>Africa, Yemen Saudi Arabia</td>
</tr>
<tr>
<td><em>S. aspera</em></td>
<td>maize, millet, sorghum upland rice, sugar cane, fonio</td>
<td>Africa</td>
</tr>
<tr>
<td><em>S. asiatica</em></td>
<td>maize, millet, sorghum upland rice, tef, sugarcane, finger millet</td>
<td>Africa, India U.S.A., China, Philippines</td>
</tr>
<tr>
<td><em>S. gesnerioides</em></td>
<td>cowpea, tobacco, sweet potato, tomato,</td>
<td>Africa, U.S.A., Saudi Arabia, Oman, Yemen, India</td>
</tr>
<tr>
<td><em>S. forbesii</em></td>
<td>sugarcane, lowland rice, maize, sorghum,</td>
<td>Africa</td>
</tr>
<tr>
<td><em>S. densiflora</em></td>
<td>cereals</td>
<td>Oman, India, Indonesia</td>
</tr>
<tr>
<td><em>S. angustifolia</em></td>
<td>cereals</td>
<td>Africa, India, Indonesia</td>
</tr>
<tr>
<td><em>S. passargei</em></td>
<td>maize, sorghum</td>
<td>Africa</td>
</tr>
<tr>
<td><em>S. klingii</em></td>
<td>sorghum, millet</td>
<td>Africa</td>
</tr>
<tr>
<td><em>S. latericea</em></td>
<td>sugarcane</td>
<td>Africa</td>
</tr>
<tr>
<td><em>S. brachicalyx</em></td>
<td>sorghum, millet</td>
<td>Africa</td>
</tr>
<tr>
<td><em>S. alzielli</em></td>
<td>sorghum, millet</td>
<td>Africa</td>
</tr>
<tr>
<td><em>S. baumannii</em></td>
<td>sorghum, millet</td>
<td>Africa</td>
</tr>
<tr>
<td><em>S. bibabiata</em></td>
<td>sorghum, millet</td>
<td>Africa</td>
</tr>
</tbody>
</table>

* Most species also parasitize wild monocots or dicots.
Scheme 1.3. Oxidation of dihydrosorgoleone (3) to quinone (4).

Scheme 1.4. Reduction of methylene blue (5) with hydroquinone structure 3 to yield the transparent reduced form (6). Autoxidation occurs on exposure to air.

Strigol (2) was the first naturally occurring germination stimulant to be isolated from root exudate of cotton (Gossypium hirsutum L.), a non-host plant (Cook et al., 1972). This sesquiterpene is hitherto the most potent Striga germination stimulant known, demonstrating activity at concentrations lower than $10^{-12}$ M. This potent
behavior is suggestive of a hormonal mode of action (Cook et al., 1972; Siame et al., 1993). However, strigol (2) is produced in low concentrations, cannot be isolated in pure form and its synthetic procedures are lengthy, requiring about 20 steps, and thus uneconomical to be of value in Striga control. In contrast to sorgoleones, Strigol (2) and its synthetic analogs (GR-compounds), are relatively stable in soils (Babiker et al., 1988; Hsaio et al., 1983 and Eplee, 1984). A number of synthetic strigol analogs have demonstrated success in vitro germination of Striga seeds (Johnson et al., 1981; Babiker et al., 1988). Other compounds which have been found to promote germination of Striga seeds include the growth hormones; kinetins, zeatin, scopletin, inositol, and inorganic compounds such as sodium hypochlorite (Worsham, 1987).

There are attendant problems as far as Striga bioassays are concerned. Notably, the germination stimulants are hydrophobic molecules and exhibit poor solubility in organic solvents. Also, Striga seeds have inherent physiological differences making it very difficult to reproduce germination data. Hitherto, the mechanism(s) of stimulation of Striga seeds by germination stimulants remains to be understood (Worsham, 1987).

1.7. QUANTITATIVE STRUCTURE-ACTIVITY (QSAR) ANALYSIS.

The wide diversity of secondary metabolites known to exist throughout the plant kingdom has been attributed to host-parasite coevolution (Erlich and Raven, 1964). The elaborate design of chemical defences in plants can involve mixtures of related structures in one biosynthetic class or multiple lines of chemical defence in a single plant species (Berenbaum, 1985). Some host cultivars (cereals and legumes) have developed means of defence against attachment by Striga spp.

To date, the reasons for differences in stimulation of germination of Striga spp. seeds by closely related compounds are not understood. To answer the question “Why is ethylene (1) or strigol (2) more potent than their congener?”, the relationship between the chemical structures and stimulatory activity of a series of naturally occurring compounds (Fig. I.1) or synthetic derivatives of strigol (2) was studied.
Quantitative structure-activity relationship (QSAR) is a complementary approach based on statistical methods for correlation analysis. QSAR lends itself to the development of models predictive of the level of stimulatory activity and even the degree of specificity of the compound under investigation. Because of the difficulty in isolating a series of related natural products large enough to allow detailed structural factors contributing to activity, QSAR studies are rare. At least five compounds are needed for each factor to be analyzed (Martin, 1978). The alternative approach of synthesizing derivatives of a prototype bioactive natural germination stimulant is hampered by the complexity of many natural products.

Chromatographic fractionation of dichloromethane (DCM) extracts (Scheme I.5), guided by assays of fractions in stimulating *Striga* spp. seed germination, revealed that the active principles were widely distributed, depending on the plant material. Our approach was both qualitative (in which the stimulatory activity was enhanced or diminished by the different functionalities in parent molecules) and quantitative (several concentrations were tested). Detailed structure-activity-relationships (SAR) were derived from the analysis of tabulated germination data which recorded the responses of *Striga* spp. seeds to a set of concentrations of a series of compounds. Because the number of concentrations ($10^{-3}$ to $10^{-20}$ M; each concentration replicated twelve times) and compounds were large, the table was complicated and therefore we had to resort to statistical methods to achieve significant data reduction and to reformat the germination data in easy-to-read forms. Germination data were normalized by transforming to arcsine and curves developed by plotting log[concentration (in Moles/L)] against arcsine transformed percentage germination.

**1.7.1. Basic Concepts of QSAR.** In an equation similar to that of Hansch (1989), the father of QSAR (Fig. I.2), we propose the following:

Stimulatory activity = $f$(Hydrophobic + Steric + Electronic effects + inhibitor effects).

Where stimulatory activity is considered to be a function ($f$) of physicochemical properties
of the compound. QSAR analysis involving Striga seeds were expressed in terms of the molar concentration of germination stimulant. Hydrophobicity is a primary factor influencing passive transport processes for germination stimulant from its source to its site of action. Moreover, hydrophobicity has been shown to be very important in regulating the interaction of bioactive compound with their bioreceptors (Compadre et al., 1990). The role of hydrophobicity in QSAR analysis has been extensively reviewed (Kabunyi, 1979; Leo et al., 1971). Steric (Compadre et al., 1990; Hansch and Klein, 1986) and electronic (Dunn, 1977) parameters have also been described. We believe that the inhibitor effects which were detected in dichloromethane extracts of dry Striga seeds (see Chapter II) affect the stimulatory capability of a given compound. The relationship between inhibitory and stimulatory activity remain to be understood. The Hansch et al. (1989) QSAR paradigm is depicted below:

I.7.2. Bioassays. Bioassays are an important research method for testing hypotheses concerning plant-parasite interactions. "Bioassays" is defined in broad terms as: any assay in which a living organism is permitted to declare whether a specific variable (e.g., presence or absence of an allelochemical, age of the plant tissue, concentration of a
germination stimulant, presence of a plant pathogen) "makes a difference" as to how the organism fares; i.e., does the variable have an impact on the plant's life history? (Leather and Einhellig, 1988). Currently, there are no guidelines on the best way to design Striga bioassay in any particular instance. The complexity of a Striga system often prohibits controlling all relevant factors; it is rare to have the total control over the genetics of Striga seeds, the environment to which these organisms have been exposed, or a complete understanding of how the testing environment interacts with the test. Such uncontrolled sources of variation often enter into an experimental design and cause errors. Since our experiment was intended to determine the stimulatory activity of different concentrations of various crude extracts and pure compounds of known structures, using a laboratory population of Striga spp. seeds was appropriate.

1.8. THE RESEARCH OBJECTIVES. Since Striga spp. seed germination activity may not necessarily be limited to any single class or even to a few classes of compounds, and since many compounds of unusual structure are known to reside in plants, this natural source appear to offer a good opportunity for obtaining new active compounds. Thus it is important to randomly collect and test several plants. Any plants whose extracts show reproducible stimulatory or inhibitory activity in the Striga germination could be re-collected in quantity and fractionated for the active agent(s). The active agents could then be evaluated like "synthetics". If these active agents satisfy certain criteria for stimulatory or inhibitory activity and chemical structural novelty they could be tested in the greenhouse; if again, they pass certain other requirements they could be tested in the field. The isolation of germination stimulants/inhibitors illustrates the usefulness of fractionation guidance by means of Striga spp. bioassays as contrasted with the classic type phytochemical procedure of isolating the constituents first and studying the biological properties later. Many of the compounds isolated in this study would have remained unknown if their presence in crude plant extracts had not been first indicated by their stimulatory/inhibitory activities. It was the original belief that the mass
fractionation effort would turn up a large number of germination stimulants/inhibitors of novel chemical structure. This hope was realized.

This study focused on improving the knowledge of the relative efficacy of known nonhost plants of *Striga* spp. which were of promising economic importance, particularly crops which were of interest to the resource-poor African farmers. Further research could then lead to devising universal and improved methods of *Striga* spp. control. This information could lead to a better understanding of what false-host crop chemical compounds are responsible for *Striga* spp. seed germination. The residues, crude extracts or purified compounds from the selected plants could possibly be used directly in *Striga* infested fields. Information obtained could also give clues about how the efficacy of other false-host crops can be predetermined through analysis of their chemical compounds. Future research could use the chemical compound information to focus either on the direct use of these compounds or on efficient breeding programs using the presence and quantity of the compound(s) as selection criteria. This study could improve the understanding of how false-host crops could be better used alone or in conjunction with other parasitic plant control practices. Finally, and most importantly, the reproducible bioassays of structure-activity studies of the pure compounds isolated at LSU could enable construction of plausible molecular mechanism(s) for germination and/or inhibition of *Striga* seeds.

The Research Objectives included:

(i). Evaluation of compounds in dry and conditioned *Striga* seeds by GC/MS.

(ii). Extraction of the plant materials from known host (for one species of *Striga*, but a false-host for another species) and suspected nonhost legume cultivars using dichloromethane (DCM) and water as solvents. The differences in the amount of germination stimulants in the extracts from leaves, stems and roots on stimulation of germination of *Striga* species seeds (*S. hermonthica, S. aspera* and *S. gesnerioides*) was investigated.
(iii). Isolation of pure compounds from the West African *Harrisonia abyssinica*. Oliv. (Simarubaceae) and the Louisiana plants of the Asteraceae family. Detailed analyses of structure- and concentration-activity relationships of the compounds isolated (Figure I.1) are discussed.

GR 24 (8)  

GR 7 (9)  

Dihydroelephantopin (10)  

Dihydroparthenolide (11)

Figure I.1. Compounds investigated for *Striga* seed germination (fig. con'd).
Isodeoxyelephantopin (12)

2-hydroxy-8\textalpha\-angeloyloxy calamene (13)

Grindelic acid (14)

Chondrillasterol (15)

(ACC) 16
Scheme 1.5. Extraction and bioassays of germination stimulants and/or inhibitors.
CHAPTER II

GC/MS EVALUATION OF COMPOUNDS IN DRY AND CONDITIONED STRIGA SEEDS
II.1. INTRODUCTION. *Striga* (Scrophulariaceae) is one of the most important genera of parasitic plants, infecting a range of major crops (e.g. sorghum, millet, maize, rice sugar cane and cowpea) in West and East Africa and Asia (Cechin and Press, 1993). The control of *Striga* spp. presents serious problems, because economically effective means of control that have been developed in the United States are either not yet available or are prohibitively expensive for the resource-poor small-scale farmers in Third World countries.

*Striga* spp. seeds have a complex germination biology which includes the need for an exogenous stimulant, which in nature, is provided by root exudates of various plants. These stimulants may not necessarily be a single substance, but may be a complex of various factors acting individually or synergistically and constituting what Schopfer (1943) called a constellation. However, the exact mechanism through which exudates or active substances from external sources induce *Striga* germination is still largely unknown.

In the absence of, or insufficiency of “conditioning”, which involves exposure to moisture in a warm environment, *Striga* spp. seeds respond either not at all or poorly to germination stimulants. *Striga* spp. seeds are sensitized to the germination stimulants by conditioning (Vallance, 1950). Several plausible explanations have been put forward to explain what happens during conditioning (Brown and Edwards, 1946; Hsiao et al., 1979; Schopfer, 1943).

Brown and Edwards (1946) hypothesized that during conditioning *Striga* seeds synthesize a substance which is the same as or similar to the stimulating substance released from plant roots. This hypothesis implies that during conditioning the amount of the substance formed in the seed itself gradually increases. The particularly interesting explanation by Schopfer (1943) states that the mere fact that *Striga* seeds require germination stimulants from an external source to initiate germination, suggests the loss of the capability of synthesizing a specific substance, which is essential for germination.
Such a loss of the capability to synthesize essential active substances is by no means uncommon in plants. Schopfer cites many examples of lower plants in which it occurs and also points out that it is met in higher plants as well. A more feasible explanation was offered by Hsiao and his collaborators (1979) who suggested that some factor(s) beneficial to the conditioning process(es) are leached out of the Striga seeds during conditioning. These germination inhibitor(s) are leached out into the conditioning liquid, without leaching out the possible germination promoters such as metabolic substrates (Vallance, 1951) or stimulants (Brown and Edwards, 1946), which had accumulated or were synthesized during conditioning.

To the best of our knowledge, no study concerning the chemical composition of dry and conditioned S. hermonthica, S. gesnerioides and S. aspera seeds has been previously done. The object of the present investigation was to shed light on the qualitative differences in CH$_2$Cl$_2$ extracts of dry and conditioned Striga spp. seeds. Preliminary identification and quantification of some of the components leached out, synthesized or retained during conditioning are reported.

II.2. MATERIALS AND METHODS

II.2.1. Seed Collection. In the United States, field and laboratory studies with Striga are done under strict quarantine regulations approved by the US Department of Agriculture. Therefore, studies involving Striga were performed at the International Institute of Tropical Agriculture, Ibadan, Nigeria. S. aspera and S. gesnerioides seeds were collected from host crops in Kano, Nigeria during the 1992 and 1993 harvesting seasons, respectively. Similarly, S. hermonthica seeds were collected from sorghum in Abuja (Nigeria) in 1993.

II.2.2. Seed Surface Disinfestation and Conditioning. The method of conditioning was adapted from Igbinnosa and Okwonko (1991) and Vasudeva Rao (1985), with minor modifications. For each experiment, 2.0 g of Striga seeds were surface disinfested by completely immersing them in 200 mL of 1 % (w/v) sodium
hypochlorite (NaOCl). Ten drops of the detergent "Tween 80" [polyoxyethylene (20) sorbitan mono-oleate] were added. After shaking, the mixture was allowed to stand for 4 min. Floating seeds were decanted and discarded. The remaining seeds were then transferred to a Buchner funnel lined with Whatman No. 1 filter paper and rinsed several times with sterile deionized water until the chlorine smell had disappeared. The filter paper containing the surface sterilized seeds was removed and the seeds air dried. The dried seeds were then conditioned on two sterile 9.0 cm diameter Whatman No. 1 filter papers, saturated with 3.0 mL sterile double-distilled deionized water, in 9.5 cm diameter petri dishes. 100 discs of Whatman GF/A) glass microfiber filter paper of 5.0 mm diameter were arranged on top of the filter paper. Seeds were carefully sprinkled on the filter disks (25-40 seeds per disc or 0.13-0.20 mg of Striga seeds per disk; approximately 200,000 seeds per gram of Striga seeds). The petri dishes were sealed with parafilm and incubated at 28°C in the dark for 10 days. Throughout the conditioning period the filter papers were kept saturated with sterile double-distilled deionized water.

II.2.3. Preparation of Extracts of Striga Seeds. Dichloromethane (CH$_2$Cl$_2$) used for sample preparation was obtained from Ibadan Chemical Co. (Oyo State, Nigeria) and double-distilled before use. Two grams of dry and conditioned seeds were extracted six times using 20 mL of CH$_2$Cl$_2$ for 24 h. The solutions from each of the six extractions were combined and evaporated under vacuum. The gummy crude extracts were shipped to the chemical laboratory at Louisiana State University and stored in the refrigerator at 4°C until analysis.

II.2.4. GC/MS Analysis. A Hewlett Packard 5971A GC/MS was used with a 5% phenyl-95% methylpolysiloxane capillary column (DB-5 ms, 12 m X 0.20 mm I.D.; 0.33 μm film thickness, J & W Scientific, Folsom, CA, USA) to separate components of extracts and obtain their electron ionization (70 eV) mass spectra. Standard stock solutions of the crude seed extracts (0.5 g) were dissolved in analytical grade CH$_2$Cl$_2$ (concentration 20 mg/mL; CH$_2$Cl$_2$ was obtained from Aldrich Chemical Company Inc.,
Milwaukee, U.S.A.). Samples (1.0 μL) from the standard stock solutions were introduced via split-injection mode (50:1) into a GC inlet at 250°C. The MS detector temperature was 250°C. The oven temperature, initially set at 40°C for 3 min, was then raised to 280°C at a rate of 20°C/min and the interface temperature was set at 280°C. Helium was used as the carrier gas and the head pressure was approximately 5 kPa. The mass range scanned was m/z 50-531 and spectra were acquired at a rate of 1.5 scans/sec. Assignments of peak identities were made by comparing their retention times with pure authentic samples isolated in our laboratory and/or by matching their mass spectra with on-line library of standard compounds (Stenhagen et al., 1974; Stenhagen et al., 1969; Sidow et al., 1970). The relative area percentages of peaks were calculated using their abundances.

II.3. RESULTS. Figures II.1-3A and B show typical chromatographic profiles of components detected in CH₂Cl₂ extracts of dry and conditioned Striga seeds, as separated on a DB-5 ms column, respectively. Several peaks were detected and their mass spectra and retention times recorded (Table II.1). The following sixteen types of compounds were identified: 1 quinone, 1 sterol, 4 fatty acids, 5 aldehydes and 5 long-chain hydrocarbons. The relative area percentages of peaks of these and the unidentified compounds gave an indication of their concentrations in Striga extracts.

The constituents common to all dry and conditioned Striga seeds were tetradecanoic acid, cis,cis-9,12-octadecadienoic acid, cis-9-octadecenoic acid and hexanal as well as an unidentified component with a retention time of 2.8 min. A comparison of dry and conditioned Striga seed extracts for the relative concentrations revealed that tetradecanoic acid, cis-9-octadecenoic acid and pentatriacontane all decreased during conditioning (Table II.1). A very dramatic increase in the relative concentration of cis,cis-9,12-octadecadienoic acid occurred during conditioning of S. aspera seeds. Conditioned S. hermonthica showed the greatest number of compounds overall and a larger number of compounds in the retention time window between 5 and 10 min (Figure
Conditioned seeds of this parasite contained trace amounts of 2,6-DMBQ and more of the compounds that were already present in dry seeds (Table II.1). For example, the relative percentage areas of cis-2-heptenal, trans,trans-2,4-decadienal, n-pentacosane, n-heptacosane and n-nonacosane of S. hermonthica seeds increased during conditioning.

The distribution patterns of compounds in S. hermonthica and S. gesnerioides seeds were similar (Figures II.1 and II.2). Their gas chromatograms indicated that the relative concentrations of cis-2-decenal, hexanal and trans,trans-2,4-decadienal increased while the level of cis,cis-9,12-octadecadienoic acid remained constant before and after conditioning. Furthermore, the seeds of both species, unlike those of S. aspera, contained hexanoic acid and 2,6-DMBQ as well as more of several unidentified components with retention time windows between 2.7 and 11 min.

Unique to dry S. gesnerioides extracts was the presence of a compound with the highest retention time of 33.4 min (Figure II.2A). S. aspera extracts contained the smallest number of compounds (Figures II.3A and II.3B). Compounds with retention times 19.4 and 19.9 were detected in extracts of dry S. aspera seeds but not in extracts of S. hermonthica and S. gesnerioides. It is worth noting that the concentration of 2,6-DMBQ in S. aspera seeds decreased during conditioning, while the most abundant component with a retention time of 19.9 min was leached out of its seeds.

In the detected hydrocarbon series (Table II.1), the heavier odd-numbered carbon n-alkanes, n-heptacosane (C\textsubscript{27}H\textsubscript{56}), n-nonacosane (C\textsubscript{29}H\textsubscript{60}) and n-pentatricontane (C\textsubscript{35}H\textsubscript{72}) predominated in the region above cis-9-octadecadienoic acid (Figures II.3.1-3). Sitosterol and 2,6-DMBQ (peaks numbers 7 and 16 in Table II.1) were assigned through comparison of the GC/MS spectra with those of authentic samples.

**II.4. DISCUSSION.** The use of CH\textsubscript{2}Cl\textsubscript{2} for the introduction of chemicals into dry seeds has opened up new possibilities of treating seeds with stimulators or inhibitors of germination (Meyer and Mayer, 1971), and it has been used in this work to extract compounds rather than introduce them. The effectiveness of CH\textsubscript{2}Cl\textsubscript{2} is presumably due
to its ability to dissolve various polar and nonpolar compounds. A GC/MS technique was selected for analysis of CH$_2$Cl$_2$ extracts because it provides identification of compounds even when the chromatographic separation is not sufficient to afford an accurate quantification (Wittkowski et al., 1981). To avoid changes in the elution order of some compounds of CH$_2$Cl$_2$ extracts (Table II.1), extraction and injection procedures as well as GC/MS conditions were standardized.

In the present investigation, it has been found that plant extracts obtained from dry and conditioned Striga seeds differ in their chemical compositions. This probably explains the variable bioassays which have been observed in various isolates of Striga species (Pepperman et al., 1982). Our results show that the relative distribution of tetradecanoic, cis,cis-9,12-octadecadienoic, cis-9-octadecenoic and other fatty acids is an important difference. The differences could be attributed to environmental factors which influence their compositions in the seeds (Stearns, 1970). In general, fatty acids are biosynthesized through a series of enzyme-mediated reactions. Acetyl-CoA carboxylase and fatty acid synthetase are the two enzymes which catalyze the de novo synthesis of saturated fatty acids (Vagelos, 1974). On the other hand, desaturase enzymes, which like fatty acid synthases are SH-enzymes (Gurr, 1974), catalyze the formation of unsaturated fatty acids. A substance targeted against the metabolism of fatty acids occurring in Striga seeds might be an effective herbicide against Striga. Specifically, these substances (herbicides) would inhibit the function of the enzymes (Ashton et al., 1994).

The fact that Striga spp. seeds synthesized compounds such as 2,6-DMBQ is fully consistent with the suggestion by Brown and Edwards (1946) that during conditioning Striga seeds synthesize a substance similar to the host-produced germination stimulant. How this is achieved is not clear from the present results. During conditioning, the seeds of S. gesnerioides synthesize several unidentified compounds with retention times 5.4, 7.2, 7.9 and 10.3 min while compounds with retention times 14.9, 17.1 and 28.5 min disappear.
The simple gas ethylene (C\textsubscript{2}H\textsubscript{4}) serves as a plant hormone with profound effects on plant growth and development (Chang and Meyerowitz, 1995). Ethylene may enhance germination of *Striga* seeds, as in other seeds, by altering the promoter-inhibitor ratio and or by promoting cell elongation during the early stages of germination process (Ketring and Morgan, 1972). However, *Striga* seed has limited capacity to convert 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate ethylene precursor, to ethylene (Babiker *et al.*, 1993). It has been shown (Yu *et al.*, 1979) that the activity of ACC synthase depends on pH, the maximal activity occurs at pH 8.5 and decreases above or below this value. We envisioned that the fatty acids detected in seed extracts possibly lowers the pH and decrease the capacity of ACC synthase to convert ACC to ethylene. During the conditioning process, the relative concentrations of tetradecanoic and *cis*-9-octadecenoic acids in *Striga* seeds decreases (Table II.1), and possibly relieves the restriction on the ethylene biosynthetic pathway.

Maillard reactions are known to reduce the activities of enzymes in dry seeds. This results in decreased metabolic capability during seed dormancy (Feeney and Whitaker, 1982; Njoroge and Monnier, 1989; Ebe *et al.*, 1983). *Striga* contains significant amounts of mannose (Nour *et al.*, 1986), a reducing sugar, which is a driving force of Maillard reactions. Since dry seeds are apparently unable to exercise repair, proteins and enzymes damaged by Maillard reaction would accumulate over time. It has been observed that soybean seed germination decrease as Maillard products accumulate (Wettlaufer and Leopold, 1991). In the case of *Striga* seeds, the Maillard products are leached out of the seeds during conditioning. Moreover, it should be noted that fatty acids in *Striga* seeds undergo free radical oxidization to hydperoxides, which can be further degraded to ketones and alcohols. Alcohols can be oxidized to aldehydes. Lipid peroxides and their secondary products can react with terminal groups of amino acids in proteins and enzymes (Feeney and Whitaker, 1982). The possibility that lipid peroxidation participates in Maillard reactions suggests that free radical oxidation and
Maillard reactions may both contribute to a common mechanism which enhances *Striga* seed viability.

The concentrations of aldehydes in *S. hermonthica* and *S. gesnerioides* seeds generally increased during conditioning. Although aldehydes have been known to occur in fruits for a long time (De Pooter, 1987), the way they are synthesized is still only solved in part. Aldehydes have been reportedly formed from heat treatment of various lipids via oxidative cleavage of the double bond (Miyake and Shibamoto, 1995; Jennings and Shibamoto, 1980). It is established that hexanal (peak number 1 in Table II.1) is formed from linoleic acid. For the biosynthesis of C3- to C5-aldehydes, circumstantial evidence points to the corresponding carboxylic acids at least in part as precursors. Aldehyde dehydrogenase is an enzyme which catalyzes the oxidation of a number of aldehydes to the corresponding acids (Asker and Davies, 1985). Also, a substance targetted against the function of aldehyde dehydrogenase might be effective in controlling *Striga*.

In comparison to other plants, *Striga* seeds contain very low levels of 2,6-DMBQ and sitosterol (Table II.1), the most ubiquitous compounds in plants (Vagelos, 1974; Heftmann, 1971; Handa *et al*., 1983; Gladu *et al*., 1991). This indicates that *Striga* species have lost the capability to synthesize these compounds as was suggested by Schopfer (1943). The significance of sitosterol in *Striga* seeds can be rationalized from several perspectives. Sitosterol is not only one of the biogenetic precursors of steroid hormones, but may have hormonal activity itself (Heftmann, 1971). Furthermore, sitosterol may act as architectural component of membranes (Nes *et al*., 1981) and function as a reserve supply from which plants can produce other sterols (perhaps including cholesterol) and progesterone (Heftmann, 1971). Sitosterol is water-soluble and might be leached out of *Striga* seeds in the field by rain or conditioning liquid in the laboratory. This might be the reason why the seeds contained very low levels of this sterol. Squalene is the precursor for synthesis of sitosterol and several enzymes are
involved in the conversion steps (Gaylor, 1974). A compound that enhances the activity of squalene enzymes might be effective in inducing "suicidal" germination of *Striga* seeds. Unpublished results at our laboratory indicate that exogenous application of sitosterol induce germination of *Striga* seeds. The level of sitosterol might be a very important parameter in evaluation of germinability of *Striga* seeds. Because germination is a risky event for any plant, especially for the tiny *Striga* seeds, one of its unique strategies is to minimize the risk of germinating by limiting the synthesis of endogenous germination stimulants (e.g. sitosterol).

The exact role of 2,6-DMBQ in *Striga* seeds is not completely understood. This quinone is not only an inhibitor of mitochondrial respiration (Chappel and Hansford, 1972; Redfearn and Whittaker, 1962) but also an haustoria-inducing principle. 2,6-DMBQ has been isolated from sorghum root (a host) extracts (Chang, 1986). Using syringaldazine, Chang and Lynn (1986) have detected laccase-type enzymes associated with the roots of *Striga asiatica* (L.) Kuntze and *Aglilinis purpurea* (L.) Raf. (Scrophulariaceae). They proposed that such enzymes released from the parasite root may cleave 2,6-DMBQ from the cell wall complex of the host which then diffuses back to *Striga asiatica* and trigger haustorial development. They concluded that zones for both *Striga asiatica* germination and haustorial induction are determined by a combination of factors including diffusion rates of promoters, the instability of active compounds, and critical threshold concentrations and exposure times required for induction. In the present study we have detected traces of 2,6-DMBQ in dry and conditioned *S. aspera* seeds as well as conditioned *S. hermonthica* and *S. gesnerioides* seeds. This is in agreement with Brown and Edwards' (1946) hypothesis of synthesis of host-produced substances by *Striga* seeds. The fact that *Striga* spp. seeds usually do not develop haustoria in the absence of exogenous compounds suggest that the level of 2,6-DMBQ in *Striga* seeds is below the threshold concentration necessary to trigger haustorial development.
In general, seed dormancy results from particular conditions prevailing internally in the seed. Several causes of dormancy have been recognized, and seeds of numerous species exhibit two or more of them simultaneously (Moore, 1979). Among the many causes of seed dormancy that have been described are (1) impermeability of seed coats to water gases, (2) immaturity of the embryo, (3) need for "after-ripening" in dry storage, (4) mechanical resistance of seed coats, (5) presence of inhibitors found either in the seed coats, dry accessory structures, or, in the case of seeds contained in fleshy fruits, in the tissues surrounding the seeds, (6) special requirement for light or its absence, and (7) requirement for chilling in the hydrated condition. The most common cause of seed dormancy in woody plants native to temperate regions is a requirement for chilling of the hydrated seeds. Such seeds commonly germinate promptly and uniformly only after they have become hydrated and exposed to low temperature (0-10°C) for a period of a few to several weeks.

It is important to note that *Striga* spp. seeds will not germinate immediately after ripening even under conditions of moisture, temperature, and oxygen tension generally favorable for growth. Such dormancy is of obvious survival value to *Striga* plants, since it tends to restrict germination to environmental circumstances suitable for seedling establishment and survival. Different *Striga* seeds in one seed plant often vary in their degree of dormancy, causing germination of the seeds to occur over an extended period of time and thus increasing the probability that at least part of them will germinate under conditions favorable for survival.

The occurrence of inhibitors in seeds is well known (Evenari, 1946) and studies in several species indicate the physiological importance of these substances (Wareing and Foda, 1956). There is evidence that in some tissues (e.g. dormant buds, potato tuber) inhibitory substances play a vital role in the regulation of dormancy (Hermberg, 1949a,b). Khan (1971) hypothesized that dormancy can result from an excess of inhibitor. However, with respect to dry *Striga* spp. seed dormancy, the presence and role
of inhibitors has hitherto been obscure. The inhibitory action might possibly be brought about by toxic concentrations of substances which are not specific germination inhibitors. We believe that the compounds leached out of *Striga* spp. seeds are germination-inhibitors while those synthesized are germination-promoters.

These high molecular weight hydrocarbons might be responsible for the "grassy" flavor (Body, 1977; Larick *et al.*, 1987) of *Striga* spp. extracts. The role of these compounds in *Striga* seeds warrant further investigation. It is important to point out that all these odd-numbered n-alkanes, as well as other even-numbered hydrocarbons have been detected in sorghum, a host plant (Kami, 1975). Generally, n-hydrocarbons develop from reaction of hydrogen-free radicals with alkyl-free radicals (hydroperoxide decomposition products) (Selke *et al.*, 1975). Our mass spectral data of n-hydrocarbons and long aliphatic chain fatty acids and aldehydes detected were confirmed by the appearance of a large number of fragments with a uniform difference of 14 mass units (Stenhagen *et al*., 1969; Silverstein and Basler, 1967). These compounds gave very intensive molecular ions (M⁺), while the ion corresponding to (M-15)⁺ was lacking. The presence of a methyl group as side chain was confirmed by the ratio of abundances (M-15)⁺/M⁺ which was about 3 (Stenhagen *et al*., 1969; Stoinova and Hadjieva, 1969). More intense clusters of peaks corresponding to C₂H₂n-1 (m/z 83, 97, 111, 125, 139, 153, etc.) in comparison to C₂H₂n+1 (m/z 85, 99, 113, 127, 141, 155, etc.) further supported acyclic olefinic nature of the unsaturated aldehydes and fatty acids detected (Silverstein and Basler, 1967).

From the present results, it is evident that one of the compounds with retention time of 19.9 min is the primary inhibitor in *S. aspera* seeds (Figure II.3A). Inhibitors detected in *S. hermonthica* and *S. gesnerioides* included heavier n-hydrocarbons (peaks 11-16 in Figure II.2A) in the retention time window between 14 and 30 min. Because germination-inhibitors may be leached out of *Striga* seeds during conditioning (Hsiao *et al*., 1979; Went, 1957), it is not unreasonable to conclude that these inhibitors may have
the potential to block stimulant-mediated germination. Furthermore, these inhibitors might constitute the (external) seed structures making them impermeable to germination stimulants. We cannot rule out the requirement for de novo synthesis of proteins essential for both germination and ethylene biosynthesis during conditioning (Babiker et al., 1993). Ultimately, the process of conditioning increases the sensitivity of Striga seeds to germination stimulants (Worsham 1987, Babiker et al., 1992).

II.5. CONCLUSIONS. In summary, our results indicate that the water-soluble endogenous inhibitors are apparently washed out of Striga spp. seeds during conditioning. Differences in relative proportions of inhibitory substances is reflected by gross manifestations of the seeds to respond to exogenously applied germination stimulants. Though we cannot quantitatively substantiate, it is clear that some compounds are also synthesized during conditioning. Future studies should emphasize investigation of the pattern of accumulation and mechanisms by which the various compounds are accumulated in Striga seeds. This will provide important information useful in designing target specific inhibitors or germination stimulants for control of Striga.
Table II.1. Relative area (%) of components in dichloromethane extracts of *Striga* seeds

<table>
<thead>
<tr>
<th>Peak&lt;sup&gt;b&lt;/sup&gt;</th>
<th>RT&lt;sup&gt;c&lt;/sup&gt;(min)</th>
<th>S. hermonthica S. gesnerioides S. aspera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dry  Con&lt;sup&gt;d&lt;/sup&gt;        Dry  Con.  Dry  Con.</td>
</tr>
<tr>
<td>Unk&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.8</td>
<td>[tr]&lt;sup&gt;f&lt;/sup&gt;  tr</td>
</tr>
<tr>
<td>1</td>
<td>3.6</td>
<td>4  60</td>
</tr>
<tr>
<td>Unk</td>
<td>5.4</td>
<td>[nd]&lt;sup&gt;g&lt;/sup&gt;  nd</td>
</tr>
<tr>
<td>2</td>
<td>5.9</td>
<td>6  26</td>
</tr>
<tr>
<td>3</td>
<td>6.1</td>
<td>tr  6</td>
</tr>
<tr>
<td>Unk</td>
<td>7.0</td>
<td>tr  5</td>
</tr>
<tr>
<td>Unk</td>
<td>7.2</td>
<td>tr  8</td>
</tr>
<tr>
<td>Unk</td>
<td>7.5</td>
<td>tr  tr</td>
</tr>
<tr>
<td>Unk</td>
<td>7.9</td>
<td>tr  tr</td>
</tr>
<tr>
<td>Unk</td>
<td>8.5</td>
<td>9  8</td>
</tr>
<tr>
<td>4</td>
<td>8.8</td>
<td>tr  37</td>
</tr>
<tr>
<td>5</td>
<td>9.1</td>
<td>6  36</td>
</tr>
<tr>
<td>6</td>
<td>9.3</td>
<td>6  52</td>
</tr>
<tr>
<td>7</td>
<td>9.7</td>
<td>nd  tr</td>
</tr>
<tr>
<td>Unk</td>
<td>10.3</td>
<td>tr  tr</td>
</tr>
<tr>
<td>Unk</td>
<td>11.2</td>
<td>nd  nd</td>
</tr>
<tr>
<td>8</td>
<td>13.3</td>
<td>69  45</td>
</tr>
<tr>
<td>Unk</td>
<td>13.6</td>
<td>tr  7</td>
</tr>
<tr>
<td>9</td>
<td>14.18</td>
<td>100 100</td>
</tr>
<tr>
<td>10</td>
<td>14.20</td>
<td>25  7</td>
</tr>
</tbody>
</table>

(Table con'd)
<table>
<thead>
<tr>
<th>Peak</th>
<th>RT&lt;sup&gt;c&lt;/sup&gt;(min)</th>
<th>S. hermonthica</th>
<th>S. gesnerioides</th>
<th>S. aspera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry</td>
<td>Con&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Dry</td>
<td>Con.</td>
</tr>
<tr>
<td>11</td>
<td>14.9</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Unk</td>
<td>15.8</td>
<td>tr</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>12</td>
<td>15.9</td>
<td>tr</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Unk</td>
<td>17.1</td>
<td>nd</td>
<td>nd</td>
<td>tr</td>
</tr>
<tr>
<td>13</td>
<td>17.3</td>
<td>tr</td>
<td>21</td>
<td>tr</td>
</tr>
<tr>
<td>14</td>
<td>19.3</td>
<td>tr</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>Unk</td>
<td>19.4</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Unk</td>
<td>19.9</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>15</td>
<td>22.4</td>
<td>40</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>16</td>
<td>27.4</td>
<td>21</td>
<td>20</td>
<td>tr</td>
</tr>
<tr>
<td>Unk</td>
<td>28.5</td>
<td>nd</td>
<td>nd</td>
<td>tr</td>
</tr>
<tr>
<td>Unk</td>
<td>33.4</td>
<td>nd</td>
<td>nd</td>
<td>12</td>
</tr>
</tbody>
</table>

<sup>a</sup> Calculated using relative abundance values (the area of the most abundant peak was assigned a value of 100%).<sup>b</sup> Numbering of peaks as shown in Figures II.1-3. Peak Identification: 1 = hexanal; 2 = cis-2-heptenal; 3 = hexanoic acid; 4 = cis-2-decenal; 5 = trans,trans-2,4-decadienal; 6 = trans,cis-2,4-decadienal; 7 = 2,6-dimethoxy-p-benzoquinone; 8 = tetradecanoic acid; 9 = cis,cis-9,12-octadecadienoic acid; 10 = cis-9-octadecenoic acid; 11 = tricosane; 12 = pentacosane; 13 = heptacosane; 14 = nonacosane; 15 = pentatriacontane; 16 = sitosterol. RT<sup>c</sup> (min) - retention time in minutes. Con<sup>d</sup> - conditioned. Unk<sup>e</sup> - not identified. [tr]<sup>f</sup> - Traces. [Nd]<sup>g</sup> - Not detected.
Figure II.1 A and B. Total ion chromatogram profiles of compounds detected in dichloromethane extracts of dry (A) and conditioned (B) *Striga hermonthica* seeds separated on DB-5ms (12m x 0.20 mm I.D.) fused silica capillary column. Numbering of peaks is as shown in Table II.1.
Figure II.2 A and B. Total ion chromatogram profiles of compounds detected in dichloromethane extracts of dry (A) and conditioned (B) *Striga gesnerioides* seeds separated on DB-5ms (12m x 0.20 mm I.D.) fused silica capillary column. Numbering of peaks is as shown in Table II.1.
Figure II.3 A and B. Total ion chromatogram profiles of compounds detected in dichloromethane extracts of dry (A) and conditioned (B) Striga aspera seeds separated on DB-5ms (12m x 0.20 mm I.D.) fused silica capillary column. Numbering of peaks is as shown in Table II.1.
CHAPTER III

ACTIVITY OF EXTRACTS FROM NONHOST LEGUMES ON THE GERMINATION OF STRIGA HERMONTICA SEEDS
III.1. INTRODUCTION. The genus *Striga* (Scrophulariaceae) comprises obligate root parasites of many graminaceous and leguminous crops. They directly affect the lives of more than 400 million people in Africa, India, and the Middle East by severely reducing the yields of various crops (Siame *et al*., 1993, Johnson *et al*., 1981). In particular, *S. hermonthica* (Del.) Benth. is a problem of enormous proportions to small-scale African farmers who make up the majority of rural communities in sub-Saharan Africa. The seeds of this parasite are tiny, around 0.30 millimeters long and 0.15 millimeters wide, and are produced in vast amounts. The parasite seeds are known to remain dormant in the soil for as long as 14 years and will germinate only after exposure to exogenous stimulating substances, usually in plant root exudates (Hauck, 1990 and Johnson *et al*., 1976).

Control methods for *S. hermonthica* include cultural procedures, hand-weeding, use of resistant crops, and biological and chemical approaches. One promising control strategy is the use of nonhost leguminous "trap crops" crops in rotation with host crops. Trap crops will cause germination of *S. hermonthica* seeds, but are not parasitized themselves. These crops induce suicidal germination and thereby reduce the *S. hermonthica* seed density in the soil. However, successful implementation of effective nonhost rotations to control *S. hermonthica* has been limited because little is known about the relative efficiency of different nonhost cultivars and the cultivar specific chemical compounds responsible for germination (Ariga and Berner, 1993).

Various techniques have been proposed for the recovery of germination stimulants (Herb *et al*., 1987). Dichloromethane (DCM) is a relatively cheap and low boiling solvent. It was used by Visser (1974) to extract germination stimulants from solutions in which roots of various hosts and nonhost plants for *S. asiatica* had been growing. A serious disadvantage of this technique of stimulant recovery is the extremely low concentration in which these substances are produced by the host plant roots. Moreover, several attempts have been made to identify the germination stimulants (Cook *et al*. 1972;
Parker, 1983), but their relative instability have hindered isolation in pure form. Investigation of different plant parts of host and nonhost legume cultivars for germination stimulants might provide additional sources of germination stimulants.

The advantages of including legumes in cropping systems have long been recognized (Nutman, 1987). The prime advantage is their ability to fix nitrogen and thus positively contribute to the nitrogen (N) balance of the cropping system. However, it must be recognized that such contributions may be of lesser significance for grain legumes than for forage legumes because of their high N harvest index (HI) and often poor nodulation (Hoshikawa, 1991). Other positive effects of legumes come from their ability to break disease cycles, improve soil physical conditions, encourage mycorrhizae, and mobilize normally unavailable soil phosphorus sources (Hoshikawa, 1991). In Africa at least, the ever-increasing demand for cereal grain mitigates against the use of grain legumes in better endowed agricultural lands and often relegates them to less favorable, usually rainfed, environments (Saxena et al., 1993). Demand for grain legumes is increasing but economics of production still do not encourage their cultivation on the more productive soils (Saxena et al., 1993). Many of the biotic and abiotic stresses faced by grain legumes contribute to the large yield gap between potential yields and realized yields as reflected in national production statistics (Johansen et al., 1994).

As part of a program concerned with investigation of leguminous nonhost cultivars as potential sources of germination stimulants, we have examined the relative efficacy in germination stimulation on S. hermonthica seeds by dichloromethane (DCM) and aqueous extracts from ten different legume cultivars. Our goal is to develop an effective S. hermonthica seed elimination or reduction control program compatible with the resource-poor small-scale farmers in developing countries.

III.2. MATERIALS AND METHODS

III.2.1. Collection of Plant Materials. All experiments were conducted at the International Institute of Tropical Agriculture, Ibadan, Nigeria. Seeds of ten different
legume cultivars (Table III.1) provided by IITA Breeding Program were planted in a greenhouse in small pots containing river sand in June 1994. The plants were uprooted after 21 days. Voucher specimens are deposited in the herbarium of IITA. Dichloromethane (DCM) and water extracts from different plant parts were analyzed for Striga stimulatory activities (Tables III.2-8 and Figures III.1-2).

III.2.2. Preparation of Solutions of GR 24 (8) and GR 7 (9). 1.5 mg of strigol analogues GR 24 (2-methyl-4-(2-oxo-2,3,3a,8b-tetrahydro-4H-indeno[1,2-b]furan-3-ylidenemethoxy)but-2-en-4-olide) (8) and GR 7 (2-methyl-4-(2-oxo-3,3a,6,6a-tetrahydro-2H-cyclopenta[b]-furan-3-ylidenemethoxy)but-2-en-4-olide) (9) (Johnson et al., 1981) were dissolved in 0.1 mL acetone and methanol, respectively. Dilution with 9.9 mL distilled sterilized water gave standard stock solutions equivalent to 1,500 mg L\(^{-1}\).

III.2.3. Dichloromethane Extraction. In the laboratory, the uprooted plants of the legume cultivars were separated into leaves, stems and roots. The plant materials were air-dried and (15 g) exhaustively extracted by soaking in 100 mL of analytical grade dichloromethane (DCM) (obtained from Aldrich Chemical Company Inc., Milwaukee, U.S.A.) at room temperature for two days, six times. The extracts from each plant part were combined, filtered by suction then evaporated below 40°C under reduced pressure to produce a thick gummy residue (ca. 0.5 g). The dichloromethane (DCM) residue (150 mg) was dissolved in 0.1 mL of 0.1 % dimethyl sulfoxide (DMSO), a solubilizing agent, and diluted with 9.9 mL sterilized distilled water to make a standard stock solutions (equivalent to 1,500 mg L\(^{-1}\)). All standard stock solutions were stored under refrigeration in crimp-top vials with Teflon seals. The solutions, undiluted or after x10 or x100 dilution, were tested for stimulatory activity on germination of conditioned S. hermonthica seeds.

III.2.4. Water Extraction. Saturated aqueous solutions were prepared by grinding 1.5 g of fresh weight of leaves, stems and roots in a mortar with 10 mL distilled sterilized
water. The extracts were filtered through Whatman No. 42 filter paper to make standard stock solutions. Solutions of lower concentrations were prepared by sequential dilution then stored and assayed for *S. hermonthica* seed germination stimulation as with the DCM extracts.

III.2.5. Isolation of Sterols from Leaves. The use of leaves in screening of legume cultivars for quantitative and qualitative differences in sterols and other germination stimulants can be a non-destructive method for assaying efficacy of plants in stimulating *Striga* spp. seed germination. Thus five legume cultivars (Table III.8) were planted in the greenhouse and their leaves collected after fourteen days. 2 g of dried and powdered leaves were extracted with 50 mL DCM, three times (24 hr each). After removal of the solvent by evaporation, the extracts (0.5 g each) were subjected to column chromatography on silica gel using EtOAc-hexane (1:10-10:1, v/v; 50 mL). The EtOAc-hexane (4:6, v/v) eluates were further rechromatographed into two fractions, 1 and 2, using EtOAc-hexane (3:7, v/v; 50 mL). Fraction 1 gave chondrillasterol (15) while fraction 2 yielded stigmasterol (17) (% yield calculated with respect to dried leaves). Identification of the individual sterols were performed by GC/MS as well as by comparing the high field $^1$H- and $^{13}$C NMR with those of reference compounds.

III.2.6. Thin-layer Chromatography (TLC). Thin-layer chromatography (TLC) (Felton, 1982; Fischer, 1991) was employed in this study to analyze the compounds present in crude dichloromethane (DCM) extracts of the different legume cultivars (Table III.2). The best separation of the germination stimulants was obtained on 0.25 mm silica gel 60F-254 precoated TLC (7 X 7 cm) plates with fluorescent indicator (EM Laboratories, No. 5760). Using capillary tubes, dichloromethane (DCM) extracts (six per plate) were applied as separate spots to one side of a TLC plate about 1.5 cm from the edge (spotting line). After sample application, the plate was placed vertically into a chamber containing developing solution of ethyl acetate/hexane (2:5) [volume ratio in parenthesis]. The spotting line was about 0.5 cm from the developing solution. After the
developing solution had moved 75% of the distance from the spotting line, to the far edge of the TLC plate, the plate was removed from the developing chamber and dried. The eluted spots, representing different compounds were visualized by spraying the plate with an acidic cobalt solution prepared by dissolving 2 g cobalt chloride (CoCl₂·6H₂O) in 100 mL of 10% aqueous sulfuric acid (H₂SO₄). The TLC plate was then heated on a hot plate to 100°C for about 1 min. The different compounds present in crude dichloromethane (DCM) extracts produced visibly colored spots whose “ratio to front” (Rf) values were determined. The Rf value expresses the position of a compound on a TLC plate. In general, Rf values in the same neighborhood suggests the presence of similar or structurally related compounds. Mathematically, Rf is a ratio of the distance that the compound moved from the spotting line, to the distance that the solvent moved from the spotting line. In principle, Rf values are between 0 and 0.999, and without units.

III.2.7. Bioassays

III.2.7.1. Crude Extracts. Seeds of one *Striga hermonthica* (Del.) Benth population collected from sorghum plants by D. K. Berner in Zaria (11.07 N, 7.44 E) in Nigeria in 1991 were used in this experiment. For each experiment, about 1440 seeds were surface disinfested and conditioned (see Materials and Methods of Chapter II). Twelve 5 mm diameter filter paper discs containing conditioned seeds (25-40 seeds per disc) were arranged in a 9.0 cm sterile petri dish lined with two (Whatman No. 1) filter papers which had been moistened with 2.0 mL sterile deionized water. For each bioassay, 360-480 seeds were used. Using a micropipette, aliquots of 600 μL of dichloromethane (DCM) or aqueous extracts were uniformly applied to each petri dish containing disks of conditioned *S. hermonthica* seeds. The petri dishes were sealed with parafilm and incubated at 28°C for 24 h. In all experiments, two reference compounds, GR 24 (8) and GR 7 (9) served as positive controls while 0.1 % (v/v) DMSO in sterile deionized water served as negative control. Germination tests were determined 24 h after application of the test solution. Three petri dishes were used as replicates for each
test and the experiments were repeated six times over a period of 3 weeks. Germinated and nongerminated seeds were counted under a binocular dissecting microscope at 20X magnification. Seeds were considered to have germinated if the radicle had emerged from the seed coat. The percentage germination was calculated for each petri dish and the germination data expressed as overall means of six experiments each replicated three times.

III.2.7.2. Sterols. Stock solutions (10⁻³ M) of chondrillasterol (15) and stigmasterol (17) were prepared using acetone as a solvent carrier while GR 24 (8), the positive control, was dissolved in methanol. The final solvent concentration was adjusted to 0.1 % (v/v). Stock solutions were refrigerated at 4°C. For each experiment, fresh solutions of lower concentrations (10⁻⁴ to 10⁻²⁰ M) were prepared by serial dilution of the stock solution and were then evaluated as germination stimulants for S. hermonthica seeds. In order to obtain statistically meaningful information on the possible action of different concentrations of sterols on germination of conditioned seeds, several treatments were performed. For each treatment, approximately 420 conditioned seeds were used and bioassayed as for crude DCM extracts. In all experiments, conditioned seeds treated with GR 24 (8) served as positive control while those treated with sterile deionized water containing 0.1 % (v/v) of acetone or methanol were used as negative controls. Each treatment was replicated twelve times over a period of 3 weeks and in each test the germination percentages were determined on three separate petri dishes. The percentage germination data reported are the mean for twelve experiments. On the basis of the mean percentage germination and the percentage viability, the relative percentage germination of S. hermonthica seeds caused by chondrillasterol (15), stigmasterol (17) and GR 24 (8) were calculated in the following way:

i). % Absolute germination = (% Germination) - (% Germination of negative control)

ii). % Relative germination = 100(% Absolute germination)/(% Viability of S. hermonthica ).
The absolute germination and viability can reach maximum values of 100% for a potent germination stimulant and highly viable seeds. The relative percentage germination at each concentration were then transformed to arc sine and examined by analysis of variance (SAS, 1989). The arc sine transformed relative percentage germination reported are expressed as a means of twelve experiments ± standard error (S. E.; shown only when larger than the symbol).

III.2.8. Statistical Analysis. Statistical comparisons were made on the amount of germination stimulant activity for *S. hermonthica* seeds by the dichloromethane (DCM) and aqueous extracts of ten legume cultivars. Germination data were transformed by square root and compared to controls using single-degree-of-freedom contrasts with the general linear models procedure of the Statistical Analysis System (SAS) programs (SAS, 1988). A significance level of $P = 0.05$ was used. Four data summaries were useful for examining the trends in percentage germination. We first calculated the mean percent germination of *S. hermonthica* seeds by dichloromethane (DCM) and water extracts from leaves, stems or roots of each cultivar. We also calculated the overall amount of germination stimulation for each cultivar using the two different extraction solvents (Tables III.3). To explain the differences in germination percentages, various interactions were analyzed including combinations of legume cultivars, legume parts, concentrations, and treatments.

III.3. RESULTS. In all our experiments, treatment of conditioned *S. hermonthica* seeds in sterile deionized water containing 0.1 % (v/v) DMSO (the negative control) did not show observable germination under the microscope. Maximum germination for most extracts was obtained using the undiluted or dilution x10 range. In many cases one or the other concentration of cultivar extracts (Tables III.4.1-3) gave higher germination than GR 24 (8) and GR 7 (9) (Tables III.2). Treatment of conditioned seeds with these strigol analogues stimulated germination in a concentration-dependent manner. In
particular, the strigol analogues resulted in high germination (71-72%) at 1,500 mg L\(^{-1}\). However, germination decreased with decreasing concentrations.

There was a definite difference in germination stimulation between the dichloromethane (DCM) and water extracts of most of the legume cultivars studied. The average percentage germination induced by dichloromethane (DCM) extracts of the various parts of *V. radiata* cv. tvr28 and *V. unguiculata* cv. iar-48 were the same (43%) and did not differ significantly from that due to *V. radiata* cv. tvr34 cultivar (44%) (Table III.3). Also, dichloromethane extracts of *V. umbellata* cv. tval and *C. cajan* cv. cits2 cultivars had the same stimulatory activity (48%). Dichloromethane (DCM) extracts of the roots of all cultivars tested contained at least a small amount of *S. hermonthica* germination stimulant activity (Tables III.4.3 and III.5.3). Of all the cultivars tested, the dichloromethane (DCM) extracts from the roots of *L. purpureus* cv. tln1 produced the highest germination (93%) which was significantly greater at x10 dilution (97%) compared to x100 dilution (44%). The undiluted extract from the roots of the other *L. purpureus* cultivar, tln28, had a greater stimulatory effect on germination (98%), but decline in activity occurred with dilution.

All of *Vigna* cultivars tested, which are not hosts of *S. hermonthica*, generally produced large amounts of stimulant activity (Tables III.4.1-3 and III.5.1-3). For example, the undiluted dichloromethane (DCM) extracts of the roots of *V. unguiculata* cv. it-84d-975 and *V. unguiculata* cv. iar-48 gave high germination (80-89%), higher germination (85-91%) at x10 dilution and low germination (23-54%) at x100 dilution. Similar trends were observed in dichloromethane (DCM) extracts of the roots of *V. umbellata* cv. tval, *L. purpureus* cv. tln1 and both cultivars of *V. radiata* and *C. cajan*. The dichloromethane (DCM) extracts of the roots of *V. umbellata* cv. wi140 gave high germination (77%), low germination (68%) and almost no response (5%) at x1, x10 and x100 dilutions, respectively. A similar trend was observed in all the dichloromethane (DCM) extracts of the other cultivar, *V. umbellata* cv. tval.
Aqueous extracts from all the cultivars studied either did not or only weakly stimulated germination of *S. hermonthica* seeds at the x10 to x100 dilution range (Tables III.5.1-3). For example, the leaves and stems of *V. umbellata* cv. wi140 and *V. umbellata* cv. tv1 were not stimulatory at all concentrations. Also, none of the aqueous extracts from either *C. cajan* cultivars stimulated germination of *S. hermonthica* seeds. This was contrary to the results with DCM. To discover the significant sources of variation in percentage germination of *S. hermonthica* seeds, we analyzed the interactions of legume cultivars, concentrations, treatment, and parts. All interactions were significant (P < 0.05). Interactions between concentrations and treatments as well at that between legume cultivars and treatments were the most significant sources of variations (Table III.6).

Numerous spots, which appeared on developed TLC plates of crude dichloromethane (DCM) extracts after treating with spray reagent, indicated that the different species and cultivars produce identical and different substance(s) that could be involved in stimulatory activity (Table III.7). It is evident that four compounds (Rf 0.10, 0.60, 0.70 and 0.77) were present in most cultivars studied. However, a different substance (Rf 0.29) was detected in the roots and stems of *C. cajan* cv. cits2. The distinct separation of spots on TLC results indicate that it is possible to isolate compounds that may have a role in *S. hermonthica* seed germination stimulation in acceptably high yields. Table III.8 lists the compositions of the sterol fractions of five cultivars. The extracts contained chondrillasterol (15) and/or stigmasterol (17) as the dominant sterol components. It is evident that the co-occurrence of both sterols have antagonistic effect in stimulation of *Striga* seeds. This prompted us to further investigate the effect of various concentrations of chondrillasterol (15) on germination of *Striga* seeds (Figures III.1 and III.2).

**III.4. DISCUSSION.** The percent germination data obtained from this study indicate that the cultivars produce chromatographically different and identical substances.
which might individually stimulate the germination of conditioned *S. hermonthica* seeds. Two types of stimulants are produced, one which is soluble in dichloromethane (DCM) but not in water, and a second type, soluble in both water and dichloromethane (DCM). The stimulatory activity in dichloromethane (DCM) extracts might be due to lactone-forming acids (Long, 1955) or related compounds. Aqueous extracts probably contain large amounts of water-soluble phenolic compounds (Lakshmi and Jayachandra, 1979) which might be responsible for any inhibitory action. Any other variations in stimulatory activity may be attributed to quantitative differences in stimulants in the dichloromethane (DCM) or water extracts. The identification of efficacious sources of *S. hermonthica* seed germinators among nonhost cultivars is highly desirable for possible use in rotations with host crops. The most important quality characteristics for utilization of a cultivar are a high content of dichloromethane (DCM) and/or water-soluble germination stimulants. *Vigna unguiculata* cv. it-81d-975 meets these requirements, having large amounts of both DCM and water extractable stimulants. Based on our germination data, it is not unreasonable to hypothesize that the *V. unguiculata* cultivars and some of the legume cultivars studied contain large amounts of germination stimulants highly related to alectrol (Muller et al., 1992). Alectrol is a highly potent germination stimulant for *Alectra vogelii* and *S. gesnerioides* and has been isolated from the root exudates of *Vigna unguiculata* (Muller et al., 1992).

![Proposed structure of alectrol](image)

The Rf values obtained from TLC experiments indicated a correlation between the presence or absence of certain compound(s) with stimulation/inhibition of germination.
TLC can be employed as a selective and non-destructive micro-detection technique to monitor the production of germination stimulants by legume cultivars throughout their life cycles. This technique could reduce the tedious *S. hermonthica* seed bioassays.

Although the germination data (Figures III.1-2) are difficult to interpret, it is evident that chondrillasterol (15) is a phytohormone (Gladu *et al*., 1991; Benveniste, 1986; Heftmann, 1971). This is encouraging because chondrillasterol (15) and stigmasterol (17) are ubiquitous in plants, and through synthetic modifications similar to those proposed by Adam and Marquardt (1986), it may be possible to design brassinolide-like substances with potent *Striga* spp. germination activities. Every steroid molecule is accessible for microbiol hydroxylation. In fact almost all the positions in a steroid molecule have been hydroxylated by various microbiol strains (Mahato and Banerjee, 1985). It is gratifying to note that several methods have been proposed for detection of steroids (Takatsuto, *et al*., 1982). Recently a new plant-growth promoting steroid, brassinolide (18), has been isolated from rape pollens (*Brassica napus* L.) (Adam and Marquardt, 1986). Brassinolide (18) is especially noteworthy because it is the first isolated natural steroid containing a seven-membered B-ring lactone and an ap configuration at the C-22 position. Furthermore, it exhibits a broad spectrum of biological activities (e.g. it promotes both cell elongation and cell division, resulting in curvature, swelling and, more dramatically, splitting of the internode in the bean second-internode bioassay and shows a strong activity in the lamina inclination assay at very low concentration) compared with known plant hormones (Yopo *et al*., 1979; Meudt *et al*., 1979). The microanalysis of brassinolide (18) and other brassinosteroids using GC/MS has been investigated and the corresponding bismethaneboronates have been found to be more suitable derivatives (Takatsuto, *et al*., 1982). Since brassinolide (18) has two vicinal diols in the side chain and the A-ring, a methaneboronate is the best derivative for GC analysis. Bismethaneboronate (19) and its analogues exhibit sharp peaks (in GC analysis) and the derivatives can be separated by chromatographic methods. The electron
ion (EI) mass spectrum of 19 shows several fragment ions. Notably, the ion at m/z 457 result from C_{23}-C_{24} fission and the strong peak at 345 result from C_{17}-C_{20} fission. The ion at m/z 155 correspond to the side-chain cleavage and is the base peak in the spectrum. The characteristic ion for a B-lactone at 332 is useful for structural determination of brassinolide analogues.

III.5. CONCLUSIONS. Further work on isolation and determination of the specific structure(s) of the germination stimulant(s) in water and dichloromethane (DCM) extracts is necessary. Bioassays of the purified compounds on Striga spp. seeds will provide invaluable information on whether they act in additive, synergistic, or antagonistic fashion. Control strategies which need to be investigated include direct application of crude or purified active products, as well as a direct incorporation of plant residues containing significant concentrations of S. hermonthica germination stimulants into infested soils (Fischer et al., 1990). This would also provide information about the soil stability of germination stimulants.
Fragments of Bismethaneboronate (19) as observed in Electron ion-mass spectrum.
Table III.1. Species and common names of legumes studied

<table>
<thead>
<tr>
<th>Species</th>
<th>Common Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vigna radiata</em> (L.) Wilczek cv. tvr28</td>
<td>Mung bean</td>
</tr>
<tr>
<td><em>V. radiata</em> (L.) Wilczek cv. tvr34</td>
<td>Mung bean</td>
</tr>
<tr>
<td><em>V. umbellata</em> (Thumb.) Ohwi &amp; Ohash cv. wi140</td>
<td>Rice bean</td>
</tr>
<tr>
<td><em>V. umbellata</em> (Thumb.) Ohwi &amp; Ohashi cv. tva1</td>
<td>Rice bean</td>
</tr>
<tr>
<td><em>V. unguiculata</em> (L.) Walp. cv. iar-48</td>
<td>Cowpea</td>
</tr>
<tr>
<td><em>V. unguiculata</em> (L.) Walp.cv. it-81d-975</td>
<td>Cowpea</td>
</tr>
<tr>
<td><em>Lablab purpureus</em> (L.) Sweet. cv. tln1</td>
<td>Lablab</td>
</tr>
<tr>
<td><em>L. purpureus</em> (L.) Sweet. cv. tln28</td>
<td>Lablab</td>
</tr>
<tr>
<td><em>Cajanu cajan</em> (L.) Huth cv. cits1</td>
<td>Pigeon pea</td>
</tr>
<tr>
<td><em>C. cajan</em> (L.) Huth cv. cits2</td>
<td>Pigeon pea</td>
</tr>
</tbody>
</table>
Table III.2. Percentage germination of conditioned *Striga hermonthica* seeds\(^a\) treated with aqueous solutions of strigol analogs, GR 24 (8) and GR 7 (9)

<table>
<thead>
<tr>
<th>Concentration (mg L(^{-1}))</th>
<th>% Germination GR 7 (9)</th>
<th>% Germination GR 24 (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,500.0</td>
<td>72 (58.1)*</td>
<td>71 (57.2)</td>
</tr>
<tr>
<td>150.0</td>
<td>71 (57.7)</td>
<td>68 (55.8)</td>
</tr>
<tr>
<td>15.0</td>
<td>64 (53.1)</td>
<td>66 (54.3)</td>
</tr>
<tr>
<td>1.5</td>
<td>60 (50.8)</td>
<td>54 (47.3)</td>
</tr>
<tr>
<td>0.15</td>
<td>53 (46.5)</td>
<td>51 (45.6)</td>
</tr>
<tr>
<td>0.015</td>
<td>50 (44.9)</td>
<td>47 (43.7)</td>
</tr>
<tr>
<td>0.0015</td>
<td>48 (43.5)</td>
<td>46 (43.2)</td>
</tr>
<tr>
<td>Mean(^a)</td>
<td>59.6 (50.7)</td>
<td>57.8 (49.6)</td>
</tr>
<tr>
<td>L.S.D(_{0.05})</td>
<td>(3.52)</td>
<td>(2.75)</td>
</tr>
</tbody>
</table>

* Figures in parentheses are arc sine transformations.

\(^a\) mean percent germination (N = 42).
Table III.3. Mean percentage germination of *Striga hermonthica* seeds\(^a\) treated with dichloromethane (DCM) or water extracts from leaves, stems and roots of legume cultivars

<table>
<thead>
<tr>
<th>Plant</th>
<th>DCM</th>
<th>Water</th>
<th>Mean(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. unguiculata</em> cv. it-84d-975</td>
<td>67 (56.3)*</td>
<td>48 (41.9)</td>
<td>(49.1) A</td>
</tr>
<tr>
<td><em>V. radiata</em> cv. tvr34</td>
<td>44 (39.2)</td>
<td>25 (23.1)</td>
<td>(31.1) B</td>
</tr>
<tr>
<td><em>L. purpureus</em> cv. tln28</td>
<td>54 (47.3)</td>
<td>8 (8.5)</td>
<td>(49.1) D</td>
</tr>
<tr>
<td><em>V. radiata</em> cv. tvr28</td>
<td>43 (37.6)</td>
<td>24 (21.4)</td>
<td>(29.5) C</td>
</tr>
<tr>
<td><em>V. unguiculata</em> cv. iar-48</td>
<td>43 (39.1)</td>
<td>17 (16.6)</td>
<td>(27.8) D</td>
</tr>
<tr>
<td><em>C. cajan</em> cv. cits1</td>
<td>62 (52.9)</td>
<td>0.01 (0.2)</td>
<td>(26.5) E</td>
</tr>
<tr>
<td><em>V. umbellata</em> cv. tval</td>
<td>48 (41.4)</td>
<td>4 (4.1)</td>
<td>(22.8) F</td>
</tr>
<tr>
<td><em>C. cajan</em> cv. cits2</td>
<td>48 (43.3)</td>
<td>0.01 (0.1)</td>
<td>(21.7) G</td>
</tr>
<tr>
<td><em>L. purpureus</em> cv. tln1</td>
<td>44 (39.1)</td>
<td>1 (1.8)</td>
<td>(20.5) H</td>
</tr>
<tr>
<td><em>V. umbellata</em> cv. wi140</td>
<td>34 (30.1)</td>
<td>5 (6.7)</td>
<td>(18.4) I</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>49 (42.6)</td>
<td>13 (12.4)</td>
<td></td>
</tr>
</tbody>
</table>

* Figures in parentheses are arc sine transformations.

\(^a\) Values are mean percentages of leaves, stems and roots (N = 54; for all plants and Water or DCM extraction, N = 540 and P = 0.0001).

\(^b\) Means associated with the same letter in a given column are not statistically different (P = 0.05).
Table III.4.1. Effect of dichloromethane (DCM) extracts from leaves of legume cultivars on germination percentages of *Striga hermonthica* seeds

<table>
<thead>
<tr>
<th>Plant</th>
<th>% Germination</th>
<th>Extract dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x1</td>
<td>x10</td>
</tr>
<tr>
<td><em>V. radiata</em> cv. tvr34</td>
<td>69 (56.1)*</td>
<td>88 (69.7)</td>
</tr>
<tr>
<td><em>V. radiata</em> cv. tvr28</td>
<td>36 (36.6)</td>
<td>55.3 (48.0)</td>
</tr>
<tr>
<td><em>V. umbellata</em> cv. wi140</td>
<td>36 (36.6)</td>
<td>0.01 (0.1)</td>
</tr>
<tr>
<td><em>V. umbellata</em> cv. tv1</td>
<td>71 (57.6)</td>
<td>54 (47.4)</td>
</tr>
<tr>
<td><em>V. unguiculata</em> cv. it-84d-975</td>
<td>91 (72.8)</td>
<td>91 (73.3)</td>
</tr>
<tr>
<td><em>V. unguiculata</em> cv. iar-48</td>
<td>25 (29.6)</td>
<td>9 (17.9)</td>
</tr>
<tr>
<td><em>L. purpureus</em> cv. tln1</td>
<td>24 (29.1)</td>
<td>65 (53.9)</td>
</tr>
<tr>
<td><em>L. purpureus</em> cv. tln28</td>
<td>82 (66.4)</td>
<td>93 (74.6)</td>
</tr>
<tr>
<td><em>C. cajan</em> cv. cits1</td>
<td>86 (68.5)</td>
<td>91 (72.9)</td>
</tr>
<tr>
<td><em>C. cajan</em> cv. c1ts2</td>
<td>71 (57.6)</td>
<td>60 (50.7)</td>
</tr>
</tbody>
</table>

* Figures in parentheses are arc sine transformations.
Table III.4.2. Effect of dichloromethane (DCM) extracts from stems of legume cultivars on germination percentages of *Striga hermonthica* seeds

<table>
<thead>
<tr>
<th>Plant</th>
<th>Extract dilution</th>
<th>% Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x1</td>
<td>x10</td>
</tr>
<tr>
<td><em>V. radiata</em> cv. tvr34</td>
<td>21 (27.3)*</td>
<td>31 (33.6)</td>
</tr>
<tr>
<td><em>V. radiata</em> cv. tvr28</td>
<td>68 (55.5)</td>
<td>38 (38.0)</td>
</tr>
<tr>
<td><em>V. umbellata</em> cv. wi140</td>
<td>85 (61.3)</td>
<td>37 (37.2)</td>
</tr>
<tr>
<td><em>V. umbellata</em> cv. tval</td>
<td>69 (56.4)</td>
<td>65 (53.6)</td>
</tr>
<tr>
<td><em>V. unguiculata</em> cv. it-84d-975</td>
<td>57 (48.9)</td>
<td>60 (50.9)</td>
</tr>
<tr>
<td><em>V. unguiculata</em> cv. iar-48</td>
<td>87 (69.2)</td>
<td>64 (53.4)</td>
</tr>
<tr>
<td><em>L. purpureus</em> cv. tln1</td>
<td>48 (43.8)</td>
<td>22 (27.4)</td>
</tr>
<tr>
<td><em>L. purpureus</em> cv. tln28</td>
<td>51 (45.5)</td>
<td>37 (37.6)</td>
</tr>
<tr>
<td><em>C. cajan</em> cv. cits1</td>
<td>50 (44.8)</td>
<td>55 (47.9)</td>
</tr>
<tr>
<td><em>C. cajan</em> cv. cits2</td>
<td>62 (52.2)</td>
<td>53 (46.4)</td>
</tr>
</tbody>
</table>

* Figures in parentheses are arc sine transformations.
Table III.4.3. Effect of dichloromethane (DCM) extracts from roots of legume cultivars on germination percentages of *Striga hermonthica* seeds

<table>
<thead>
<tr>
<th>Plant</th>
<th>Extract dilution</th>
<th></th>
<th></th>
<th></th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x1</td>
<td>x10</td>
<td>x100</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>V. radiata</em> cv. tvr34</td>
<td>48 (43.6)*</td>
<td>80 (63.4)</td>
<td>51 (45.8)</td>
<td>(50.9)</td>
<td></td>
</tr>
<tr>
<td><em>V. radiata</em> cv. tvr28</td>
<td>46 (42.7)</td>
<td>81 (64.0)</td>
<td>50 (45.0)</td>
<td>(50.5)</td>
<td></td>
</tr>
<tr>
<td><em>V. umbellata</em> cv. wi140</td>
<td>77 (61.3)</td>
<td>68 (55.3)</td>
<td>5 (12.6)</td>
<td>(43.1)</td>
<td></td>
</tr>
<tr>
<td><em>V. umbellata</em> cv. tval</td>
<td>69 (56.1)</td>
<td>80 (63.3)</td>
<td>11 (19.4)</td>
<td>(46.2)</td>
<td></td>
</tr>
<tr>
<td><em>V. unguiculata</em> cv. it-84d-975</td>
<td>89 (71.2)</td>
<td>91 (72.3)</td>
<td>54 (47.2)</td>
<td>(63.6)</td>
<td></td>
</tr>
<tr>
<td><em>V. unguiculata</em> cv. iar-48</td>
<td>80 (63.5)</td>
<td>85 (67.3)</td>
<td>23 (28.6)</td>
<td>(53.1)</td>
<td></td>
</tr>
<tr>
<td><em>L. purpureus</em> cv. tln1</td>
<td>93 (74.9)</td>
<td>97 (80.8)</td>
<td>44 (41.7)</td>
<td>(65.8)</td>
<td></td>
</tr>
<tr>
<td><em>L. purpureus</em> cv. tln28</td>
<td>98 (81.9)</td>
<td>79 (62.6)</td>
<td>26 (30.6)</td>
<td>(58.4)</td>
<td></td>
</tr>
<tr>
<td><em>C. cajan</em> cv. cits1</td>
<td>85 (67.0)</td>
<td>90 (71.7)</td>
<td>49 (44.4)</td>
<td>(61.0)</td>
<td></td>
</tr>
<tr>
<td><em>C. cajan</em> cv. cits2</td>
<td>65 (53.7)</td>
<td>69 (56.4)</td>
<td>23 (28.5)</td>
<td>(46.2)</td>
<td></td>
</tr>
</tbody>
</table>

* Figures in parentheses are arc sine transformations.
Table III.5.1. Effect of aqueous extracts from leaves of legumes on germination percentages of *Striga hermonthica* seeds

<table>
<thead>
<tr>
<th>Plant</th>
<th>x1</th>
<th>x10</th>
<th>x100</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. radiata</em> cv. tvr34</td>
<td>76</td>
<td>72</td>
<td>0.01</td>
<td>(39.7)</td>
</tr>
<tr>
<td><em>V. radiata</em> cv. tvr28</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>(0.1)</td>
</tr>
<tr>
<td><em>V. unguiculata</em> cv. wi140</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>(0.1)</td>
</tr>
<tr>
<td><em>V. unguiculata</em> cv. tva1</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>(0.1)</td>
</tr>
<tr>
<td><em>V. unguiculata</em> cv. it-84d-975</td>
<td>80</td>
<td>75</td>
<td>11</td>
<td>(47.6)</td>
</tr>
<tr>
<td><em>V. unguiculata</em> cv. iar-48</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>(0.1)</td>
</tr>
<tr>
<td><em>L. purpureus</em> cv. tln1</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>(0.2)</td>
</tr>
<tr>
<td><em>L. purpureus</em> cv. tln28</td>
<td>47</td>
<td>0.01</td>
<td>0.01</td>
<td>(10.7)</td>
</tr>
<tr>
<td><em>C. cajan</em> cv. cits1</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>(0.1)</td>
</tr>
<tr>
<td><em>C. cajan</em> cv. cits2</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>(0.2)</td>
</tr>
</tbody>
</table>

* Figures in parentheses are arc sine transformations.
### Table III.5.2. Effect of aqueous extracts from stems of legumes on germination percentages of *Striga hermonthica* seeds

<table>
<thead>
<tr>
<th>Plant</th>
<th>x1 (%)</th>
<th>x10 (%)</th>
<th>x100 (%)</th>
<th>Mean (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. radiata</em> cv. tvr34</td>
<td>21 (26.5)*</td>
<td>0.01 (0.2)</td>
<td>0.01 (0.2)</td>
<td>(8.9)</td>
</tr>
<tr>
<td><em>V. radiata</em> cv. tvr28</td>
<td>71 (57.3)</td>
<td>39 (38.5)</td>
<td>0.01 (0.1)</td>
<td>(32.0)</td>
</tr>
<tr>
<td><em>V. umbellata</em> cv. wi140</td>
<td>0.01 (0.1)</td>
<td>0.01 (0.1)</td>
<td>0.01 (0.1)</td>
<td>(0.1)</td>
</tr>
<tr>
<td><em>V. umbellata</em> cv. tval</td>
<td>0.01 (0.1)</td>
<td>0.01 (0.2)</td>
<td>0.01 (0.1)</td>
<td>(0.1)</td>
</tr>
<tr>
<td><em>V. unguiculata</em> cv. it-84d-975</td>
<td>52 (45.9)</td>
<td>17 (24.1)</td>
<td>0.01 (0.1)</td>
<td>(23.4)</td>
</tr>
<tr>
<td><em>V. unguiculata</em> cv. iar-48</td>
<td>50 (45.0)</td>
<td>62 (52.2)</td>
<td>14 (21.9)</td>
<td>(39.7)</td>
</tr>
<tr>
<td><em>L. purpureus</em> cv. tln1</td>
<td>7 (15.0)</td>
<td>0.01 (0.1)</td>
<td>0.01 (0.1)</td>
<td>(5.1)</td>
</tr>
<tr>
<td><em>L. purpureus</em> cv. tln28</td>
<td>28 (31.9)</td>
<td>0.01 (0.1)</td>
<td>0.01 (0.1)</td>
<td>(14.6)</td>
</tr>
<tr>
<td><em>C. cajan</em> cv. cits1</td>
<td>0.01 (0.1)</td>
<td>0.01 (0.1)</td>
<td>0.01 (0.1)</td>
<td>(0.1)</td>
</tr>
<tr>
<td><em>C. cajan</em> cv. clts2</td>
<td>0.01 (0.2)</td>
<td>0.01 (0.1)</td>
<td>0.01 (0.1)</td>
<td>(0.1)</td>
</tr>
</tbody>
</table>

* Figures in parentheses are arc sine transformations.
Table III.5.3. Effect of aqueous extracts from roots of legumes on germination percentages of *Striga hermonthica* seeds

<table>
<thead>
<tr>
<th>Plant</th>
<th>x1</th>
<th>x10</th>
<th>x100</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. radiata</em> cv. tvr34</td>
<td>47 (43.3)*</td>
<td>10 (18.3)</td>
<td>0.01 (0.2)</td>
<td>(20.6)</td>
</tr>
<tr>
<td><em>V. radiata</em> cv. tvr28</td>
<td>69 (56.3)</td>
<td>41 (39.3)</td>
<td>0.01 (0.1)</td>
<td>(32.1)</td>
</tr>
<tr>
<td><em>V. umbellata</em> cv. wil40</td>
<td>13 (21.2)</td>
<td>25 (30.3)</td>
<td>2 (8.0)</td>
<td>(19.8)</td>
</tr>
<tr>
<td><em>V. umbellata</em> cv. tval</td>
<td>34 (35.9)</td>
<td>0.01 (0.2)</td>
<td>0.01 (0.1)</td>
<td>(12.1)</td>
</tr>
<tr>
<td><em>V. unguiculata</em> cv. it-84d-975</td>
<td>77 (61.6)</td>
<td>80 (63.2)</td>
<td>40 (39.2)</td>
<td>(54.7)</td>
</tr>
<tr>
<td><em>V. unguiculata</em> cv. iar-48</td>
<td>24 (29.2)</td>
<td>0.01 (0.1)</td>
<td>0.01 (0.1)</td>
<td>(9.9)</td>
</tr>
<tr>
<td><em>L. purpureus</em> cv. tln1</td>
<td>0.01 (0.1)</td>
<td>0.01 (0.1)</td>
<td>0.01 (0.1)</td>
<td>(0.1)</td>
</tr>
<tr>
<td><em>L. purpureus</em> cv. tln28</td>
<td>0.01 (0.1)</td>
<td>0.01 (0.1)</td>
<td>0.01 (0.1)</td>
<td>(0.1)</td>
</tr>
<tr>
<td><em>C. cajan</em> cv. cits1</td>
<td>0.01 (0.1)</td>
<td>0.01 (0.1)</td>
<td>0.01 (0.1)</td>
<td>(0.1)</td>
</tr>
<tr>
<td><em>C. cajan</em> cv. c1ts2</td>
<td>0.01 (0.1)</td>
<td>0.01 (0.1)</td>
<td>0.01 (0.2)</td>
<td>(0.1)</td>
</tr>
</tbody>
</table>

* Figures in parentheses are arc sine transformations.
Table III.6. Source of variation in percentage germination of *Striga hermonthica* seeds as explained by the correlations ($r^2$) of legume cultivars, legume parts, concentrations and treatments

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>% variation explained ($r^2 \times 100$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Legume cultivars (ten)</td>
<td>9.2*</td>
</tr>
<tr>
<td>Legume parts (Leaves, stems and roots)</td>
<td>35.6</td>
</tr>
<tr>
<td>Concentrations (dilutions x1, x10 and x100)</td>
<td>20.4</td>
</tr>
<tr>
<td>Treatments (DCM and water)</td>
<td>28.8</td>
</tr>
<tr>
<td>Legume cultivars $\times$ Legume parts</td>
<td>21.9</td>
</tr>
<tr>
<td>Legume cultivars $\times$ Concentrations</td>
<td>30.3</td>
</tr>
<tr>
<td>Legume cultivars $\times$ Treatments</td>
<td>47.7</td>
</tr>
<tr>
<td>Legume parts $\times$ Concentrations</td>
<td>22.6</td>
</tr>
<tr>
<td>Legume parts $\times$ Treatments</td>
<td>34.3</td>
</tr>
<tr>
<td>Concentrations $\times$ Treatments</td>
<td>54.7</td>
</tr>
</tbody>
</table>

* Figures are arc sine transformed.

$\times$ interaction.
Table III.7. Thin-layer chromatography (TLC) mobilities of compounds in dichloromethane (DCM) extracts

<table>
<thead>
<tr>
<th>Crop</th>
<th>Crop part</th>
<th>( R_f ) values(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V. radiata )</td>
<td>Roots</td>
<td>0.64, 0.77, 0.88</td>
</tr>
<tr>
<td>cv. tvr34</td>
<td>Stem</td>
<td>0.10, 0.40, 0.60, 0.70, 0.88</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.60, 0.77</td>
</tr>
<tr>
<td>( V. radiata )</td>
<td>Roots</td>
<td>0.10, 0.77, 0.84, 0.89</td>
</tr>
<tr>
<td>cv. tvr28</td>
<td>Stem</td>
<td>0.70, 0.77, 0.84</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.23, 0.70, 0.84</td>
</tr>
<tr>
<td>( V. umbellata )</td>
<td>Roots</td>
<td>0.10, 0.77, 0.86, 0.89</td>
</tr>
<tr>
<td>cv. tvv1</td>
<td>Stem</td>
<td>0.10, 0.74, 0.88</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.10, 0.12, 0.88</td>
</tr>
<tr>
<td>( V. umbellata )</td>
<td>Roots</td>
<td>0.60, 0.77</td>
</tr>
<tr>
<td>cv. wi140</td>
<td>Stem</td>
<td>0.64, 0.77</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.10, 0.40, 0.60, 0.70, 0.88</td>
</tr>
<tr>
<td>( V. unguiculata)</td>
<td>Roots</td>
<td>0.60, 0.70, 0.84, 0.90, 0.97</td>
</tr>
<tr>
<td>cv. it-84d-975</td>
<td>Stem</td>
<td>0.65, 0.70, 0.77, 0.84, 0.90</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.77, 0.88, 0.97</td>
</tr>
<tr>
<td>( V. unguiculata)</td>
<td>Roots</td>
<td>0.10, 0.88</td>
</tr>
<tr>
<td>cv. iar-48</td>
<td>Stem</td>
<td>0.10, 0.70, 0.88</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.77, 0.82, 0.88, 0.97</td>
</tr>
<tr>
<td>( L. purpureus )</td>
<td>Roots</td>
<td>0.60, 0.77, 0.88</td>
</tr>
<tr>
<td>cv. tln1</td>
<td>Stem</td>
<td>0.10, 0.64, 0.77, 0.88</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.10, 0.40, 0.60, 0.70, 0.88, 0.92</td>
</tr>
</tbody>
</table>

(Table con’d)
<table>
<thead>
<tr>
<th>Crop</th>
<th>Crop part</th>
<th>R&lt;sub&gt;f&lt;/sub&gt; values&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. purpureus</em> cv. tln28</td>
<td>Roots</td>
<td>0.60, 0.70, 0.77, 0.81</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>0.69, 0.77, 0.83</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.10, 0.44, 0.60, 0.70, 0.78, 0.81</td>
</tr>
<tr>
<td><em>C. cajan</em> cv. cits1</td>
<td>Roots</td>
<td>0.60, 0.29, 0.70, 0.77, 0.93</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>0.70, 0.83</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.10, 0.70, 0.81</td>
</tr>
<tr>
<td><em>C. cajan</em> cv. cits2</td>
<td>Roots</td>
<td>0.29, 0.43, 0.84</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>0.29, 0.54, 0.86</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.10, 0.60, 0.68, 0.70, 0.81, 0.89</td>
</tr>
</tbody>
</table>

<sup>a</sup> TLC, Silica gel; EtOAc-hexane, 2:5.
Stigmasterol (17)

TLC; \( R_f: \) 0.60

GC/MS; RT: 26.10

Chondrillasterol (15)

\( R_f: \) 0.40

25.70

**Table III.8. Composition (%) of sterols isolated from leaves**

<table>
<thead>
<tr>
<th>Legume cultivar</th>
<th>% Chondrillasterol (15)</th>
<th>% Stigmasterol (17)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vigna radiata</em> cv. tvr34</td>
<td>0</td>
<td>52</td>
</tr>
<tr>
<td><em>V. umbellata</em> cv. wi140</td>
<td>66</td>
<td>31</td>
</tr>
<tr>
<td><em>Lablab purpureus</em> cv. tln1</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td><em>L. purpureus</em> cv. tln28</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td><em>Cajanus cajan</em> cv. cits1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>C. cajan</em> cv. cits2</td>
<td>0</td>
<td>40</td>
</tr>
</tbody>
</table>

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
Fig. III.1. Effect of chondrillasterol (15) on germination of conditioned *Striga hermonthica* seeds occurring in Bida (A) and Kano (B) locations in Nigeria. Data are arc sine transformed relative percentage germination expressed as means ± S. E. of twelve replicates (P = 0.05). Control is GR 24 (8) dissolved in methanol (fig. con’d).
Fig. III.1. Effect of chondrillasterol (15) on germination of conditioned *Striga hermonthica* seeds collected in Abuja location in Nigeria during the 1992 (C) and 1993 (D) harvesting seasons. Data are arc sine transformed relative percentage germination expressed as means ± S. E. of twelve replicates (P = 0.05). Control is GR 24 (8) dissolved in methanol.
Fig. III.2. Effect of chondrillasterol (15) on germination of conditioned *Striga aspera* seeds occurring in Kano (E) location in Nigeria. Data are arc-sine transformed relative percentage germination expressed as means ± S. E. of twelve replicates (P = 0.05). Control is GR 24 (8) dissolved in methanol.
CHAPTER IV

*IN VITRO* GERMINATION OF *STRIGA HERMONTHICA* AND *S. ASPERA* SEEDS BY 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID (ACC)
IV.1. INTRODUCTION. Effects of various solutions of pure compounds on germination of *Striga* spp. seeds has been the central focus of our research. Thus it is appropriate to define the term “seed germination”. Seed germination occurs when the growing seed tissue pierces or breaks the seed coat, including the endosperm and/or pericarp. Therefore, the ease of seed germination depends upon the growth potential of the seed tissue and/or the mechanical resistance of the seed coat. Seeds display two basic types of dormancy: (1) primary or embryo dormancy due to the failure of seed tissues to grow, seed immaturity, or secondary dormancy due to the gradual loss of the growth capacity of seed tissues during a period of water imbibition; (2) coat-imposed dormancy due to the mechanical restriction of the seed coat. It is possible that only the complete loss of the mechanical restriction of the seed envelope allows seed germination, with no need to alter the active growth potential of seed tissues. In the case of *Striga* spp., many researchers have tried to reduce the mechanical restraint of the seed coat by means of various synthetic and natural germination stimulants which include, kinetin (Williams, 1961), zeatin (Worsham *et al.*, 1959), gibberellic acid (Cook *et al.*, 1972, 1966) scopoletin (Worsham *et al.*, 1962) thiourea and allylthiourea (Brown and Edwards, 1945), sulfuric acid (Egley, 1972), sodium hypochlorite (Hsiao *et al.*, 1981) and ethylene (Bebawai and Eplee, 1986 and references therein). Although it has been shown that ethylene is the most effective germination stimulant for *Striga* spp. (Egley and Dale, 1970), treatment of field soil with ethylene gas is not an economical option for the small-scale African farmers.

ACC (16) was first isolated and characterized from cider apples and perry pears by Burroughs (1957) and synthesized by Ingold *et al.* (1922). ACC (16) is an example of cyclopropane amino acids which are hypoglycemic and toxic. Several biologically active compounds contain this moiety. For example, the chrysanthemum acids are constituents of the insecticidal pyrethrins, sirenin is the sex pheromone of the water mole *Allomyces*, presqualene pyrophosphate is an important intermediate in the biosynthesis of
triterpenes and sterols, and cycloartenol may be a precursor of phytosterols (Goad, 1970; George and Kalyanasundaram, 1994; Law, 1971). It should be noted that in plants, ACC (16) is present in relatively low amount and exposure to some types of plant stress can increase ACC (16) and, ultimately, ethylene synthesis (Hoffman et al., 1983).

From the structural standpoint, ACC (16) possesses no asymmetric carbon and hence lacks enantiomers. For a clearer understanding of its chemistry, it is instructive to consider briefly the molecular orbital representation of the cyclopropane ring. The carbon atoms are believed to be hybridized in such a way that their orbitals have greater \( p \) character than in normal \( sp^3 \) bond. The two orbitals from any one carbon lie in the same plane at an angle of 104° to each other and, as a result, the C-C bonding orbitals are not directed towards each other so the bonds are described as "bent" or "banana" (Christie, 1970). Bonds in the cyclopropane ring, therefore, differ markedly from those in alkanes or higher alicyclic compounds with undistorted bond angles, and cyclopropane compounds have properties which are similar in many ways to those of alkenes. For example, they undergo addition reactions with electrophilic reagents but with simultaneous ring opening. The cyclopropane ring is resistant to mild oxidative procedures including those commonly used to locate double bonds, such as ozonolysis or permanganate-periodate fission.

To our knowledge, there are no published results on the laboratory testing of the Nigerian Striga hermonthica (Del.) Benth. and S. aspera (Willd.) Benth. seeds using 1-aminocyclopropane-1-carboxylic acid (ACC) (16). In the present study, the effect of various concentrations of ACC (16) (ranging from \( 10^{-3} \) M to \( 10^{-20} \) M) on germination of these two Striga spp. seeds were examined. In addition, the various mechanisms which have been proposed for the chemical and biological oxidation of ACC (16) to generate ethylene are discussed.
IV.2. MATERIALS AND METHODS

IV.2.1. General. *S. hermonthica* (Del.) Benth seeds were collected from sorghum, in Bida (1993), Abuja (1992 and 1993), and Kano (1991) locations in Nigeria. *S. aspera* seeds were collected in Kano (1993) location. ACC (16) and the strigol analogue, GR 24 (8) were supplied by D. K. Berner.

IV.2.2. Viability Test. Viability of *Striga* spp. seeds was estimated using tetrazolium red (2,3,5-triphenyltetrazolium chloride) test (Hsiao et al., 1979). Approximately 400 seeds were placed on a clean sheet of filter paper in a petri dish. The filter paper was then saturated with 1.0% tetrazolium red solution (pH 7). The petri dish was sealed and incubated at 35°C for 5 days. Embryos of viable seeds stained pink when observed under a microscope. Very pale pink and unstained embryos indicated non-viable seeds. Viability experiments were repeated eight times and the mean percentage viability was determined. The total percent viability (V) was calculated on the basis of the formula: \[ V = \frac{G}{T} \times 100 \]

\[ G \] is the number of viable seeds and \[ T \] is total number of seeds.

IV.2.3. Preparation of ACC (16) and GR 24 (8). Because ACC (16) is very soluble in water, stock solutions (10^-3 M) were prepared using sterile deionized water. GR 24 (8), the positive control, is hydrophobic and stock solutions (10^-3 M) were prepared using methanol as a solvent carrier. The final methanol concentration was adjusted to 0.1% (v/v). Solutions were refrigerated at 4°C. For each experiment, fresh solutions of lower concentrations (10^-4 to 10^-20 M) were prepared by serial dilution of the stock solution and were then evaluated as germination stimulants for *Striga* spp. seeds as described in Chapter III (see Materials and Methods).

IV.3. RESULTS. Viabilities (V) for *S. hermonthica* isolates from Bida, Abuja (1992), Abuja (1993) and Kano (1991), were determined to be 87, 74, 73 and 70% respectively. For *S. aspera* isolate from Kano (1993), V was determined to be 66%. The effect of ACC (16) concentrations on the germination of conditioned *S. hermonthica* and
S. aspera seeds is summarized in Figures IV.1-2. Compared to GR 24 (8), ACC (16) did not show substantial differences in stimulation of germination. Both compounds caused germination stimulation which did not show linear correlation with concentration. The treatment of conditioned Striga spp. seeds with negative control (sterile deionized water) elicited no stimulatory activity. The isolate of S. hermonthica occurring in Bida location showed both the least and the greatest seed germination responses to ACC (16) and GR 24 (8), respectively. Furthermore, germination of this isolate did not significantly respond to ACC (16) at concentrations lower than 10^{-13} M. Conditioned S. aspera seeds showed both the greatest and least germination response at 10^{-12} M and 10^{-17} M, respectively. It should be noted that the concentration range in our experiments was broad and the solutions were two to five times more dilute than those employed by Babiker et al. (1993).

IV.4. DISCUSSION. Babiker and coworkers (1993) observed that ACC (16) alone did not stimulate germination or ethylene production by S. asiatica seeds. According to them, the inability of S. asiatica seeds to convert ACC (16) into ethylene was suggested by lack of response to ACC (16) at the concentrations used (5 to 200 mM) and is an indication of low ethylene forming enzyme (EFE) activity. A limited ability to convert ACC (16) into ethylene has been associated with dormancy of several seeds which require ethylene for germination (Corbineau et al., 1989; Satoh et al., 1984). In our experiments, the low percentage germination observed in conditioned seeds of S. hermonthica and S. aspera when treated with ACC (16) suggested the presence of low levels of EFE (Babiker et al., 1993; Jackson and Parker, 1991). Logan and Stewart (1991) have indicated that ACC (16) at or below 100 mM did not induce germination or ethylene production and that a very high concentration (50 mM) was needed to give germination figures close to those attained by sorghum root exudates. This response to ACC (16) may be due to inhibition of root elongation by the high concentration of ethylene generated (Abeles et al., 1972; Eliasson, 1989). It is tempting to speculate that
although some *Striga* spp. may have a limited capacity to convert ACC (16), the immediate ethylene precursor, to ethylene, exogenous introduction of 16 to conditioned *Striga* spp. seeds possibly triggers the activation and/or synthesis of EFE and consequent conversion to ethylene (Adams and Yang, 1979).

**IV.4.1. Mechanisms of Formation of Ethylene from ACC (16).** The main biological source of ethylene in plants is methionine (20) and it has been established that ACC (16) is the immediate precursor of ethylene biosynthesis (Yang and Hoffman, 1984). The synthesis of ethylene is regulated by two different types of factors. One type is promoted by internal signals, as ethylene plays an important role in some developmental stages, e.g. seed germination (Ketring and Morgan, 1972; Logan and Stewart, 1991; Stewart and Press, 1990), root and leaf growth (Chadwick and Burg, 1970), leaf abscission (Kao and Yang, 1983), and flower and fruit senescence (Burg, 1968). Ethylene enhanced in the developmental stage is recognized as “auxin induced” ethylene. Ethylene induced under stress conditions is defined as “stress ethylene” (Yang and Hoffman, 1984). Very pronounced stress ethylene is produced when the plant is under environmental stresses, e.g. water stress (Hoffman et al., 1983), chilling stress (Field, 1984), wounding (Hoffman and Yang, 1982), the exposure to SO$_2$ (Meyer et al., 1987), ozone (Langebartels et al., 1991) or other pollutants (Fuhrer, 1982).

The enhancement of ethylene production, together with the changes in concentrations of other kinds of plant hormones, provides the plants with the mechanisms in adapting or avoiding the environmental stress. Quite a few papers have been published about the changes in the concentrations of ACC (16) in stressed plant tissues. Many observations demonstrate that the level of 16 in stressed plants increases many times more than that under normal conditions (Kende and Boller, 1981; Elstner et al., 1985; Rodecap and Tingey, 1986). Treatment of conditioned *S. hermonthica* and *S. aspera* seeds with ACC (16) possibly relieves this stress. Although the present study was not aimed at investigating the mechanism of action of ACC (16), the germination data
suggested a hormonal type of action (Lockwood and Brain, 1976). Various mechanisms showing how ACC (16) stimulate the biosynthesis of ethylene are depicted in Schemes IV.1-4.

Adams and Yang (1979) postulated a mechanism to account for the formation of ethylene (Scheme IV.1). The first step is the activation of methionine (20) by adenosine triphosphate (ATP) to produce S-adenosylmethionine (SAM) (21), which then reacts with pyridoxal enzyme (in Scheme IV.1, pyridoxal phosphate stands for pyridoxal enzyme) to form a Schiff's base (22). It is well known that pyridoxal enzymes are capable of catalyzing γ-elimination (1,3-elimination) of the proton from the α-carbon of an amino acid yielding a carbanion (Davis and Metzler, 1972). Because the positive sulfonium group of SAM (21) is an excellent leaving group, once the carbanion is formed, an intramolecular nucleophilic displacement reaction can occur, resulting in the elimination of 5'-methylthioadenosine (MTA) and the formation of ACC (16). The carboxyl, C-1, C-2, and C-3 of 16 are derived, respectively, from C-1, C-2, C-3, and C-4 of methionine (20). The enzyme mediating the conversion of ACC (16) to ethylene has not been isolated and characterized. However, the characteristics of the in vivo reaction strongly suggest that the enzyme is membrane-associated, labile and disrupted by various treatments (Yu et al., 1979). The conversion process is oxygen-dependent and is inhibited by Co²⁺, temperatures above 35°C, light and uncouplers such as dinitrophenol (Yu et al., 1979). Based on the enzymic reaction catalyzed by this enzyme (ACC synthetase ?) it can be classified as SAM-MTA-lyase (α,γ-eliminating).

IV.4.2. Hypochlorite Chemistry. For satisfactory and reproducible germination data, the use of a specific concentration of sodium hypochlorite (NaOCl) is critical. Striga spp. seeds disinfested with solutions of different concentrations of NaOCl for one to several minutes showed differences in germination response to the positive control, GR 24 (8) (data not shown). NaOCl is effective as a disinfecting and sterilizing agent against a broad range of bacteria, viruses, and fungi (Mercer and Somers, 1957). It is
commonly used in pre-treatment for seeds in Europe and North America (Guthrie, 1978), particularly for seeds which may be excessively contaminated with saprophytic fungi (Knudson, 1950). The chemical is used to eradicate those surface-borne organisms which may interfere with germination. NaOCl has a strong oxidizing property which makes it highly reactive with amino acids (Kantouch, 1971), nucleic acids, amines, and amides (Hayatsu et al., 1971). The general reaction between amino acids and NaOCl produces the respective aldehyde, NH$_4$Cl and CO$_2$ (Kantouch, 1971).

Hiyama et al. (1975) found that substituted cyclopropylamines react with NaOCl or other oxidants to form ethylene and other products. For example, 1-phenylecylopropylamine (26) is oxidized via nitrenium ion intermediate (27) to yield ethylene and benzonitrile (Scheme IV.2). Pirrng and McGeehan (1983) suggested ring opening of the nitrenium ion (27) to afford the ketimine (28) (Scheme IV.3). According to them, hydrolysis of 28 yields 2-oxo-3-butenoic acid (29) which cyclizes to $\alpha$-Ketobutyrolactone (30). Support for the processes in scheme IV.3 was obtained by following the oxidation of [1-$^{13}$C, $^{15}$N] ACC (16) in an NMR spectrometer. The $^{15}$N atom split the enriched C-1 signal in its $^{13}$C NMR spectrum. The signal at 37.8 ppm appears as a doublet, with a coupling constant of 8.7 Hz. Addition of NaOCl to the solution results in a new doublet appearing at 122.8 ppm with a larger coupling constant (18.5 Hz) (Route A). This coupling is characteristic of cyanide groups and is assigned to the cyanide group of the cyanoformic acid. Moreover, oxidation of labeled ACC (16) with N-chlorosuccinimide result in appearance of strong singlets at 207, 142, and 97 ppm (Route B). These, they assigned to the 2-keto group of 29, C-3 of (31) (the enol of $\alpha$-ketobutyrolactone), and C-2 of (32) (the hydrated form of (29)), respectively.

It is well known that NaOCl reacts with $\alpha$-amino acids with the formation of $N$-chloroamine as an intermediate which is ultimately degraded into aldehyde, ammonia, and CO$_2$ (Schonberg and Moubacher, 1952). In keeping with the results of Hiyama et al. (1975), Lizada and Yang (1979) developed a sensitive assay for ACC (16), in which the
amino acid is treated with sodium hypochlorite in the presence of mercuric chloride, resulting in 80% yield of ethylene (Scheme IV.4). Cupric ions also catalyzed the reaction. In the absence of the divalent ions the yield of ethylene was only 13%. The C-1 and the carboxyl group of ACC (16) were converted to oxalic acid via its monoamide (33).

IV.4.3. Possible Basic Mechanisms of Action of Ethylene on Germination of Striga Spp. Seeds. The biological effects of ethylene are well known (Pratt and Goeschl, 1969). There are three ways in which ethylene might stimulate germination of Striga spp. seeds: (1) by serving as a cofactor in some reaction; (2) by being oxidized to some essential component and being incorporated into tissue; or (3) by binding to a receptor, providing some essential function, and then either diffusing away or being destroyed as is the case with other hormones. Each of these ways has been investigated (Beyer, 1979; Abeles et al., 1972). However, the initial events that occur at the ethylene receptor site(s) which cause these effects are entirely unknown. Likewise, the location, chemical nature, and the type of bonding which occurs between the receptor site(s) and ethylene (e.g., covalent, coordinate, van der Waals) and the subsequent molecular changes, if any, that ethylene undergoes during receptor site activation are unknown. Burg and Burg (1967) have proposed on the basis of indirect evidence that ethylene binds to a metal-containing receptor site. Deuteration, especially where the deuterium is directly bound to an unsaturated carbon atom as in ethylene, increases the stability of silver-ion olefin complexes (Jones, 1968). A basic question concerning the primary mechanism of ethylene action is whether ethylene acts catalytically or whether it is permanently incorporated into the tissue during mechanism of action. Conflicting results have appeared in the literature as to the extent of incorporation when radioactive ethylene is applied to fruit tissues. Jansen (1963, 1964) found that mature green avocados and green pear fruits exposed to 1 ml/L of 14C-labeled ethylene for several days incorporated 0.05% ethylene of the applied radioactivity. In other experiments, however, Buhler et al. (1957)
failed to obtain any incorporation of radioactivity into ripe oranges, limes, papayas, green apples, tomatoes, and grapes. Abeles et al. (1972) suggested that covalent bonding is important in ethylene action. Based on their results deuterated ethylene had the same physiological effect as protonated ethylene suggesting that C-H bonds were not broken. Furthermore, isotopic discrimination experiments did not make the deuterated ethylene ($\text{C}_2\text{D}_4$) approximately half as effective as its lighter analogue ($\text{C}_2\text{H}_4$). There is no support that hydrogen bonding might be important in ethylene action. It appears that the forces which bind ethylene to its site of action must be of the weak van der Waals type (Abeles et al, 1972).

From the structural point of view, two of the $sp^2$ orbitals of each carbon atom of ethylene overlap with the $ls$ orbitals of the two hydrogen atoms to form two strong $\sigma$ C-H bonds, while the third $sp^2$ orbital of each carbon atom is used to form a strong $\sigma$ C-C bond between the two carbons. The two unhybridized $2p$ orbitals of the two carbon atoms are parallel to each other and can themselves overlap to form a molecular orbital (the $\pi$ orbital) spreading over the two carbon atoms and is situated above and below the plane containing the carbon and hydrogen atoms. The electron occupying this molecular orbital are called $\pi$ electrons. The carbon atoms are drawn closer together by the $\pi$ bond. The lateral overlap of the $p$ orbitals that occurs in forming a $\pi$ bond is less effective than the linear overlap that occurs in forming a $\sigma$ bond, and the $\pi$ bond is less effective than the $\sigma$ bond, as well as being different in position (Sykes, 1965).

The direct chemical reaction of ethylene with an enzyme, a structural protein, nucleic acid or other cell components is a possible basic mechanism of action of ethylene. The most reactive portion of ethylene is, of course, the double bond, for the distribution of the $\pi$ electrons in two layers, above and below the molecule and extending beyond the C-C bond axis, means that a region of negative charge exists to attract any electron-seeking (i.e. oxidizing) agents. Because the two electrons in the $\pi$ orbital are less firmly held between the carbon nuclei, they are more readily polarizable than those of the $\sigma$
bond. Additions to ethylene can proceed through ionic mechanisms, as the π electrons become polarized, either by approaching agent or other causes, as follows:

This type of process tends to predominate in polar solvents, while free radical additions predominate in non-polar solvents, particularly in the presence of other radicals and light. Ethylene is an effective free radical scavenger (Stahmann, 1968). Water, a polar solvent, is the major constituent of living organisms, and most biochemical reactions occur in aqueous solutions. Also, membranes of the cell have a high proportion of proteins and phospholipids, both polar in nature. Neutral lipids make up a small proportion of the membranes, but constitute the majority of fat deposits of oil globules in plant cells.

Ethylene, with its high solubility in both lipids and water, could be expected to be found in all these locales, both polar and non-polar. Because polar solvents for ethylene predominate in the cell, one would expect ionic reactions to be quantitative and most important, with the electrophilic reagents attacking the double bonds. However, it must be kept in mind that a quantitatively unimportant reaction may be qualitatively significant, because of the chain reactions it sets in motion.

Certain metallic ions might fill the role of an electrophilic reagent in an attack on the double bond. Suppose, for example, that a metal M is part of an oxidoreductase or other electron transferring agent, and that it could be reversibly oxidized and reduced between two states, $M^{++}$ and $M^{+}$. For the sake of simplicity, we shall assume that the electron acceptor is oxygen, although it could well be an intermediate electron carrier that would either directly or through other electron carriers transfer its electrons to oxygen. We envision a series of reactions (scheme IV.5) similar to that proposed by Jones (1968).
In its oxidized state the metal is assumed to attack the double bond, but not in its reduced state (Scheme IV.5). The affinity of ethylene for R-M^{++} is much greater than for R-M^{+}. After the metal removes an electron from ethylene, the now positively charged carbon on the ethylene can take up an electron from a donor (or could form a bond with a nucleophilic reagent). For these reactions to continue, since the amount of compound R-M in the system is probably limited in typical biological fashion, R-M^{+} must give up its electrons. Ethylene is also regenerated in its initial form. For an increase in respiratory rate in the presence of trace amounts of ethylene, the ethylene-R-M complex, if it exists, must complete the process of taking up and giving up its electrons more rapidly than does R-M alone. Large amounts of ethylene might crowd out the oxygen (if it were the immediate acceptor of electrons from R-M), preventing access to the M^{+} site on M-R and thus slowing down electron transfer. This probably explain the observed decline in respiration after the climacteric peak in both respiration and ethylene production. The mechanism would also explain why low oxygen tensions reduce the efficacy of ethylene in producing some of its physiological effects. Another factor to consider is that certain concentrations of carbon dioxide act as competitive inhibitors of ethylene in at least some of its effects. The fact that the concentrations required are high may also be linked to the crowding concept. The affinity of CO_{2} for R-M^{+} would have to be much less than that of ethylene, if it were so. On the other hand, CO_{2} could have its effect through an entirely different mechanism. However, the grossly observable physiological effects result from complex interweavings of reactions; and their interpretation in terms of action of one substance is premature to our knowledge.

No direct evidence for this basic mechanism of action (Scheme IV.5) of ethylene exists at present, but perhaps the outline of it will stimulate the search for it. In principle, there are a number of metal containing-compounds involved in electron transfer in living systems, not all of them react with ethylene with equal ease. The influence on the metal of the R group as well as of the properties of the metal itself are involved in determining
the reactivity; and little information exists on this influence, where biological compounds are concerned.

It is also important to consider the chemicals surrounding ethylene in the cell. For example, lipids also have double bonds, and free radical reactions with ethylene are a possibility. What other changes can one expect to take place in the ethylene molecule itself when it accomplishes its biological functions? One thinks first, in these days of allostery, of conformational changes, where the same group of atoms can assume different arrangements without the breaking of any bonds. However, it is known that there is resistance to rotation about the carbon-carbon double bonds. The reason for this is the overlap of the \(2p\) atomic orbitals forming the \(\pi\) bond, and thus the strength of the \(\pi\) bond, will be at maximum when the two carbons and four hydrogen atoms are exactly coplanar. It is only in these position that the \(\pi\) atomic orbitals are exactly parallel to each other and capable of the maximum overlapping. Any disturbance of this coplanar state by twisting about the \(\sigma\) bond joining the two carbon atoms would lead to reduction in \(\pi\) overlapping and hence a decrease in the strength of the \(\pi\) bond: it will thus be resisted. Even if one does not expect the conformational changes in ethylene itself, one should keep in mind that other substances attracted by the ethylene itself, may alter their conformation. With enzymes, conformational changes often accompany changes in reactivity.

**IV.5. CONCLUSION.** There are many potential mechanisms of formation of ethylene from ACC (16) as an intermediate and also for the action of ethylene in biological systems. To thoroughly explore these possibilities it will take the combined efforts of many specialists, including chemists, physicists, horticulturalists, physiologists, biochemists and pathologists. The knowledge gained thereby will also have application in many fields, most foreseeable of which is the control of the noxious *Striga* spp. seeds.
Scheme IV.1. Mechanism of Adams and Yang (1979) for the biosynthesis of ethylene.
Scheme IV.2. Formation of ethylene by the chemical oxidation of 1-phenylcyclopropylamine (26) (Hiyama et al., 1975).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
Scheme IV.4. Proposed mechanism of Lizada and Yang (1979) for the oxidation of ACC with hypochlorite.
Electron from biological oxidations

oxidized electron carrier

reduced electron carrier

$\text{2 } \text{R-M}^{++}$

$\text{2 } \text{R-M}^{+}$

$\frac{1}{2} \text{O}_2$

$2 \text{H}^+$

$\text{H}_2\text{O}$

Scheme IV.5. A Possible mechanism of action of ethylene.
Fig. IV.1. Effect of ACC (16) on germination of isolates of conditioned *Striga hermonthica* seeds occurring in Bida (A) and Kano (B) locations in Nigeria. Data are arc sine transformed relative percentage germination values expressed as means ± S. E. of twelve replicates (P = 0.05). Control is GR 24 (8) in methanol (fig. con'd).
Fig. IV.1. Effect of ACC (16) on germination of isolates of conditioned 
*Striga hermonthica* seeds collected in Abuja location in Nigeria 
during the 1992 (C) and 1993 (D) harvesting seasons. Data are arc 
sine transformed relative percentage germination values expressed 
as means ± S. E. of twelve replicates (P = 0.05). Control is GR 24 (8) 
in methanol.
Fig. IV.2. Effect of ACC (16) on germination of isolate of conditioned *Striga aspera* seeds collected in Kano location (E) in Nigeria during the 1993 harvesting season. Data are arc sine transformed relative percentage germination values expressed as means ± S.E. of twelve replicates (P = 0.05). Control is GR 24 (8) in methanol.
CHAPTER V

STIMULATION OF STRIGA HERMONTHICA GERMINATION BY 11βH,13-DIHYDROPARTHENOLID (DHP)
V.1. INTRODUCTION. Plant-derived chemicals are of considerable agronomic interest because of their low level of hazard to nontarget plant species (Duke et al., 1987). Strigol (2), a natural product isolated from the root exudates of a nonhost, cotton (Gossypium hirsutum L.), has proven to be particularly effective in stimulating the germination of witchweed seeds (Cook et al., 1972). It has been found that application of 10⁻¹¹ M solutions of strigol (2) result in over 50% germination of S. asiatica seeds (Kendall et al., 1979). Thus much effort has been made to explore the potential of synthetic strigol analogues in inducing Striga spp. seed germination. These strigol analogues are also being used as reference compounds in evaluation of other strigol related compounds. However, the difficult and expensive syntheses (Kendall et al., 1979; MacAlpine et al., 1976; Johnson et al., 1981) as well as the requirement of many different types of chemical plant equipment (Vail et al., 1990) makes the use strigol (2) and its analogues prohibitively expensive for control of Striga in small-scale farming.

Sesquiterpene lactones (SLs) which are ubiquitous in nature appear to be attractive candidates in control of Striga spp.

Sesquiterpene lactones (SLs) constitute a large and diverse group of secondary metabolites, which are common constituents of several plant families. However, the greatest number of these bitter principles have been reported in the family Asteraceae with over 3500 reported structures (Fischer, 1991; Picman, 1986; Seaman, 1982). It is generally accepted that the SLs are derived from \textit{trans,trans}-farnesy1 pyrophosphate (34) by initial cyclization process and subsequent oxidative biomodifications (Fischer, 1991). SLs can exhibit a broad spectrum of biological activities including antibiotic, phytotoxic, allergenic and plant growth regulatory properties (Picman, 1986). Previous reviews (Fischer et al., 1979; Seaman, 1982) have discussed the chemistry and taxonomic significance of sesquiterpene lactones. Many other studies (Picman, 1986; Kupchan, 1970) have shown that the biological activities of SLs can be mainly attributed to the \(\alpha\)-methylene-\(\gamma\)-lactone (35) moiety and that this structural feature is almost indispensable.
for this action (Kupchan et al., 1971). Structures containing this functional group have been reported to alkylate by conjugate addition to biological nucleophiles (Hall et al., 1977).

The sesquiterpene lactone dihydroparthenolide (DHP, 11), has been isolated (Fischer et al., 1981) in multi-gram quantities from Ambrosia artemisifolia (common world-wide ragweed) and identified (Parodi et al., 1987). Dihydroparthenolide (DHP, 11) occurs in significant concentrations (0.15% of weight) in Louisiana populations of A. artemisifolia and has been previously investigated for S. asiatica germination stimulatory activity (Fischer et al., 1989; 1990). This present study was undertaken in order to develop improved S. hermonthica control procedures based on the concept of suicidal germination by DHP (11). To facilitate concentration-activity comparisons, various aqueous solutions of 11 (isolated at the LSU laboratory by Fischer et al., 1981, 1989) were prepared using acetone and methanol as carriers.

V.2. MATERIALS AND METHODS

V.2.1. General. All bioassays were done at the International Institute of Tropical Agriculture, Ibadan, Nigeria. Seeds of S. hermonthica were collected in 1993 by D. K. Berner from sorghum plants in Bida, Nigeria. A pure sample of DHP (11) was isolated (Fischer et al., 1981) in multi-gram quantities from Ambrosia artemisifolia (common ragweed) and identified (Parodi et al., 1987) at the LSU laboratory. GR 24 (8) was provided by D. K. Berner. The viability (Hsiao et al., 1979) of S. hermonthica seeds was determined to be 87%.

V.2.2. Seed Conditioning. For each experiment, about 1440 S. hermonthica seeds were surface disinfested by completely immersing in 3.6 mL of 1 % (w/v) sodium hypochlorite (NaOCl) to which three drops of the detergent "Tween 80" [polyoxyethylene (20) sorbitan mono-oleate] were added. After shaking, the mixture was allowed to stand for 4 min. Floating seeds were decanted and discarded. The remaining seeds were transferred to a Buchner funnel lined with Whatman No. 1 filter paper and rinsed several
times with sterile deionized water until the chlorine smell disappeared. The filter paper containing the surface disinfested seeds was removed and the seeds air dried. The disinfested, dried seeds were conditioned on 100 glass fiber disks (of 5.0 mm diameter) arranged on top of two sterile Whatman No. 1 filter papers in 9.5 cm diameter petri dishes. *S. hermonthica* seeds (25-40 seeds per disk) were carefully sprinkled on each glass fiber disk. The petri dishes were sealed with parafilm and incubated at 28°C in the dark for 10 days. Throughout the conditioning period the filter papers were saturated with sterile deionized water.

**V.2.3. Measurement of Germination.** Because of the low water solubility of the DHP (11) and GR 24 (8) aqueous stock solutions were prepared using acetone and methanol as carriers. Two sets of stock solutions of 10⁻³ M DHP (11) were prepared by dissolving 2.5 mg in 0.1 mL acetone or methanol and diluted with 9.9 mL distilled water to obtain the desired concentration. 10⁻³ M GR 24 (8) was similarly prepared by dissolving 2.98 mg in 0.1 mL methanol before diluting with 9.9 mL distilled water. The stock solutions were refrigerated at about 4°C. For each experiment, fresh solutions of lower concentrations were prepared by serial dilution of stock solutions and evaluated as germination stimulants for conditioned *S. hermonthica* seeds. Twelve 5.0 mm diameter glass fiber disks containing the conditioned seeds were arranged in a 9.0 cm sterile petri dish lined with two (Whatman No. 1) filter papers which had been moistened with 2.0 mL sterile deionized water. Using a micropipette, aliquots of 600 µL of test solutions were uniformly applied to each petri dish containing disks of conditioned *S. hermonthica* seeds. The petri dishes were sealed with parafilm and incubated at 28°C for 24 h. In all experiments, conditioned *S. hermonthica* seeds treated with sterile deionized water containing 0.1% (v/v) acetone or methanol served as negative controls. Germination tests were determined 24 h after application of the test solution. Because of the difficulties in reproducing *Striga* bioassays, it was necessary to replicate each test in three separate petri dishes per experiment and repeat the experiments twelve times over a period of 3 weeks.
For each test, approximately 360 conditioned seeds were used. Germinated and nongerminated seeds were counted under a binocular dissecting microscope at 20X magnification. Seeds were considered to have germinated if the radicle had protruded from the seed coat. The germination data were transformed to arc sine (Gomez and Gomez, 1984) and processed by analysis of variance (SAS, 1989).

V.2.4. Molecular Modeling. Energy-minimized geometries and conformational energies (enthalpies) required by the major conformers of DHP (11), strigol (2), and GR 24 (8) were calculated using a PCMODEL molecular mechanics program developed by Burkert and Allinger (1982). This program uses the MMX force field. The van der Waals volumes (VDWs) and the dipole (DP) moments were also calculated. As there are presently no data from which it is a priori possible to choose the conformer(s) to use as a model for DHP (11) active in stimulating germination of conditioned S. hermonthica seeds, the energy minimization procedure was carried out starting from every conformer that could be formed using Dreiding models. Conformers 11a and 11b are modelled by homologues of DHP (11). MM calculations of conformation of strigol (2) were carried out using the coordinates of X-ray crystallographic analyses by Coggon et al. (1973). In the case of the synthetic strigol analogue GR 24 (8), the coordinates of strigol (2) were used in energy minimization calculations except in the A-ring. The entropy terms have not been explicitly considered in the present work, but differences in the entropy contributions for the molecules in the series investigated should largely be compensated by differences in hydrophobic binding (Liljefors et al., 1985).

V.3. RESULTS

V.3.1. Germination Data. The curves of means of arc sine transformed relative percentage germination of S. hermonthica seed germination plotted against the log [concentration] of GR 24 (8), the positive control, and DHP (11) are shown in Figure V.1. Neither compound inhibited germination at concentrations as high as 10^{-3} M. GR 24 (8) in methanol as a carrier exhibited high stimulation effects (54-79%) on seed
germination at all concentrations. Solutions of DHP (11) prepared in acetone exhibited a broader range of seed germination stimulation (19-75%) than those in methanol (32-64%), and paradoxically, the highest stimulation of germination (75%) at $10^{-20}$ M. Solutions of DHP (11) in methanol showed two seed germination “plateaus” between $10^{-5}$ to $10^{-6}$ M and $10^{-15}$ to $10^{-16}$ M concentration range, respectively. Unlike DHP (11) in acetone, GR 24 (8) elicited a single seed germination “plateau” between $10^{-13}$-$10^{-14}$ M. It is of interest to note that while $10^{-9}$ M solutions of DHP (11) in acetone and methanol showed the lowest seed germination stimulation activities of 19 and 32%, respectively, a $10^{-9}$ M solution of GR 24 (8) elicited a high germination of 71%.

V.3.2. Molecular Mechanics Calculations. The results of MM calculations are shown in Table V.1. The conformational energy, van der Waals volume and dipole moment of strigol (2) were calculated to be 43.71 kcal/mol, 2.35 and 6.38, respectively. The energy of the most thermodynamically stable conformer (8) was determined to be 45.37 kcal/mol. The energy of conformer DHP (11a) with H-13 in $\alpha$-orientation was within 1.48 kcal/mol of the energy of the thermodynamically most stable conformer. The interconversion between the two conformers may thus be described as a large amplitude torsional motion. However, the energy barrier for the interconversion of 11 to 11b (Me-14 in $\alpha$-orientation) was calculated to be large (104.04 kcal/mol).

V.4. DISCUSSION. The germination data indicated variable seed germination stimulatory activities by DHP (11) and GR 24 (8) which can be attributed to structural differences. GR 24 (8) possesses an aromatic A-ring and contains all of the features of strigol (2) with the exception of both of the gem-dimethyl groups and the introduction of formal double bonds in the 4,5 and 6,7 positions. In addition, GR 24 (8) does not have a hydroxyl group at the 4-position as does strigol (2). Other structural similarities of 8 and 2 that merit comment include the butenolide ring structure (D-ring of strigol) joined to another methyleneoxy bridge. The fact that butenolides undergo alkaline hydrolysis or...
degradation during storage, leading to variable results in *Striga* bioassays (Pepperman *et al.*, 1982; Hassanali, 1984) appears to be a major limitation for strigol and its analogues. On the other hand, the structure of DHP (11) is unique in several respects. Firstly, it has a germacranolide skeleton (36) with a 4,5-epoxide of a 1(10), 4(5)-cyclodecadiene system. Secondly, it has C(14) and C(15) lying syn on the β-face of the medium ring. Thirdly, it does not contain the common alkylation α-methylene-γ-lactone moiety (35), which is generally considered to be essential for biological activities (Picman, 1986). However, the epoxide ring at the 4,5-position can be opened by transannular cyclization generating a cationic center at C-10 which can be a receptor for biological nucleophiles. It should be noted that 11 is stable at room temperature and its lactone ring is similar to the butenolide ('D') ring of strigol (2), except that it lacks the double bond (Fig. V.1). Previous studies has revealed a positive correlation between general phytotoxicity and the presence of the C-13 exocyclic methylene cyclopentenone groups (Rodriguez, 1977). The absence of these functionalities in DHP (11) makes it an attractive nontoxic candidate in *Striga* control.

The germination data demonstrated that DHP (11) can promote germination at concentrations lower than $10^{-15}$ M. These activities are considerably higher than the activities of other natural compounds reported to affect seed germination (Duke, 1986), and suggests that DHP (11) may be a representative of a new class of plant growth hormones (Cook *et al.*, 1972; Kalsi *et al.*, 1977; Johnson *et al.*, 1976). We hope these results can be extrapolated to greenhouse and eventually, field experiments. It is reasonable to assume that the rain washes can readily transport DHP (11) from the source plant (crop residue or decomposing litter) into the soil where they can easily attain concentration levels applied in our study. It has been demonstrated that iso-alantolactone (37) can persist in mineral soil and organic soil for 3 months (Picman, 1987), supporting the assumption that DHP (11) may persist in the soil at least for 3 to 7 days, sufficient time to stimulate *Striga* spp. seed germination.
The failure of 10^{-9} M solutions of DHP (11) to induce significant germination may be interpreted as a lack of intrinsic hormone-like activity and that the ability to stimulate the endogenous ethylene biosynthesis probably failed at this concentration. Alternatively, this could be viewed as the poor penetration or metabolic inactivation of conditioned S. hermonthica seeds by the 10^{-9} M solutions. Further work is needed to establish the factors responsible for this behavior.

V.4.1. Conformational Analyses. While NMR studies have shown that strigol (2), GR (8) and DHP (11) the stable conformations at room temperature (Cook et al., 1972; Johnson et al., 1981; Fischer et al., 1989), it is not certain that they are the only forms populated since the NMR method is unable to distinguish between a single "fixed" conformation or a mixture of conformations in rapid equilibrium resulting an averaged symmetry equivalent to that of the single conformation. For the latter possibility, a much lower temperature would be required to slow down the residual process on the NMR time scale. To solve this enigma, the utilization of molecular mechanics (MM) calculations on the conformational analysis of germination stimulants is inevitable (Osawa et al., 1989; Shimazaki et al., 1991 and references therein).

Ten-membered-ring system of the germacrane skeleton of DHP (11) is flexible (Fischer et al., 1979). Furthermore, it is well established that the conformational freedom is greatly reduced in the cyclic structures, and this is where the possibility exists in developing method for exhaustive conformer generation (Goto and Osawa, 1989). Analysis of the conformers of DHP (11) indicated that there are at least two conformers (11 and 11a) which are candidates for the biologically active structure (within the context of our "stimulant-receptor interaction model" proposed below). Our interest was to compare the minimum energies of DHP (11) with those of the leading germination stimulants, strigol (2) and its analogue, GR 24 (8) (Table V.1). Significantly high stimulatory activities of compound 2 may be attributed to electrostatic effects of the functionalities and also intra- and intermolecular hydrogen-bonding between strigol (2)
molecules and receptor (Lijefors et al., 1985). At the moment, the only way we can reconcile the relatively low stimulatory activity of DHP (11), as compared to GR 24 (8) is that it exists in a mixture of conformers with large energy variations.

V.4.2. The Stimulant-Receptor Interaction Model. We propose the presence of receptor sites in Striga seeds in which the reactive sites of germination stimulants attach and induce the production of ethylene. Plausible features of receptor models may include: (1) the use of the entire strigol (2) molecule in the construction of the models instead of parts of the molecule; (2) the receptor has a specific and probably flexible van der Waals volume; (3) the use of the complete structure of different conformers in the calculations of conformational energies; and (4) addition of flexibility to the model with respect to the required location of the actiphore of the germination stimulants. Furthermore, we assume the receptor sites are complementary to a wide variety of functional groups including the double bond, lactone moiety, hydroxyl, and/or methyl groups. The natural strigol (2) is thus very useful in defining spatial relationships in the cavity of the receptor active site between positions of molecular parts crucial for full stimulatory activity (Liljefors et al., 1985). The corresponding molecular arrangements in the studied conformers (Table V.1) may, through conformational changes, be positioned in these space locations. The energy required for such a conformational change probably correlates with the stimulatory activity of the conformer. A low conformational energy should correspond to a high biological activity and vice versa. It should be noted that at the present stage of development our proposed model is applicable to the natural strigol (2) and its analogues as well as sesquiterpene lactones which have the capability to position the crucial molecular parts, as defined above, in the required positions. Our stimulant-receptor interaction model should be understood as a model for an “activated complex” related to the efficacy (intrinsic stimulatory activity) rather than as a model for the initial binding of the substrate. In terms of stimulant-receptor binding energies, this model implies that strigol (2) and its analogues have very similar interaction energies with the assumed
receptor sites. It must also be borne in mind that the exact details of the germination mechanism are yet to be proven experimentally and that the mechanism may be a complex multistep process as shown in Scheme V.1. It is necessary for a germination stimulant to mimic the natural stimulant, possibly strigol (2) or its analogues (Hauck et al., 1992), with respect to the spatial locations of the actiphore. The high activity of strigol (2) compared to its synthetic analogue GR 24 (8) indicate that not only the gem-dimethyl groups but also the hydroxyl group (OH-4) may efficiently interact with a hydrophobic region of the assumed receptor cavity, a region which is complementary to van der Waals surface of the substrate molecule. Thus, the shape of the part of the receptor cavity interacting with the A, B, C, and D rings of strigol (2) may be a deep “groove” or “pocket,” at least fully circumscribing the actiphore (rings C and D).

The different stimulatory activities of the biologically active compounds strigol (2) and DHP (11) and GR 24 (8), are, according to our stimulant-receptor interaction model, due to the differences in binding capabilities to the receptor, or to form an “activated complex” with the receptor. This is reflected in the differences in the conformational energies (Table V.1). We believe that the differences in energies between the lowest conformational energy structures (thermodynamically favorable) and higher energy conformations correspond to the energies required to bring the molecules from their preferred conformations to their biologically active conformations. The probability of a germination stimulant binding to the receptor sites of enzymes in *Striga* seeds probably depends on the energy of the conformationally rearranged structure relative to that of the thermodynamically preferred one. Gratifyingly, the van der Waals volumes of the natural strigol (2) and GR 24 (8) are comparable but differ from that of DHP (11) (Table V.1). The extra volume required by compound GR 24 (8) relative to strigol (2) is probably not tolerated by the receptor site and this is reflected by their overall performances in stimulation of *S. hermonthica* seeds (Figure V.1).
V.4.3. **Solvents.** The relative stimulatory activity of DHP (11) and GR 24 (8) in germination of *S. hermonthica* seeds generally depended upon the concentration and further modulated by the carrier systems used. This may account for the large differences in the percentage germination by a wide range of concentration tested. The stimulatory activity may also be associated with the ease of penetration and subsequent metabolic conversion of the germination stimulant to one active functional group. Alternatively, intercalation of each substance at a critical concentration directly into the membrane systems of conditioned *S. hermonthica* seeds may be the most relevant factor, as for the alcohols (Taylorson, 1988). Organic solvents have been used for extraction and dissolving natural stimulants (Brown *et al.*, 1951). It was known that the compounds to be tested as germination stimulants, DHP (11) and GR 24 (8) are hydrophobic, and solutions of known concentrations would be difficult to obtain without the use of a carrier system. The purpose of the carrier is to solubilize the germination stimulant prior to dilution with water. The influence of carrier system on stimulatory activities on *Striga* spp. seed germination was perceptible (Fig. V.1). As the concentrations of the compounds and the solvents decreased, the percentage germination fluctuated. We envisioned that the observed germination at concentrations below $10^{-14}$ M, was largely due to the solvents employed. Increases in seed activity germination stimulation may have been due to solvent polarity (e.g. acetone versus methanol) suggesting the existence of different conformations in equilibrium (Bhatt *et al.*, 1965). Djerassi *et al.* (1960) have shown that in passing from a relatively non-polar to polar medium, the proportion of the conformer(s) with higher dipole moment increases. It is not unreasonable to hypothesize that in general methanol-induced respiration and hence *Striga* spp. seed germination rate is higher than acetone-induced. Moreover, methanol treatments have been reported to greatly increase productivity and/or water requirements of desert crops (Nonomura and Benson, 1992). Methanol in small quantities is a natural product of plant metabolism (Zhang *et al.*, 1993; Hansen *et al.*, 1994; Cossins, 1964; McDonald and Fall, 1993), but
the mechanism by which methanol affects growth and water use efficiency is hitherto unknown. Methanol is known to accumulate in maturing seeds (Obendorf et al., 1990), probably as a product of pectin demethylation by the enzyme pectin methylesterase (PME). The presence of methanol during germination has been reported (Vancura and Stotzky, 1976; Taylorson, 1979). Non-viable seeds have low levels of methanol due to a decrease in pectin methylesterase activity (Obendorf et al., 1990). In this study methanol was used to dissolve DHP (11) and GR 24 (8) at much lower concentrations than would naturally occur.

By soaking seeds of a number of plant species in acetone for six months, Milborrow (1963) demonstrated that acetone is not toxic and does not reduce viability. He further showed that acetone-soluble and not water-insoluble compounds penetrate seeds and could be recovered from the seedlings. These findings suggest that acetone employed in dissolving germination stimulants acted primarily as a solvent carrier and had no adverse effects on the seeds.

V.4.4. Plausible Mechanism of Stimulation of Germination of *Striga hermonthica* Seeds by DHP (11). With the success of the laboratory bioassays and the attendant promise of using DHP (11) in *Striga* control, it becomes important to understand the molecular mechanistic basis of its stimulatory activity. To the best of our knowledge, there have been no mechanistic proposals or even speculation regarding the ways in which DHP (11) might stimulate the germination of *Striga* seeds. Several unusual “maxima” and “minima” of percentage germination (Table V.1) suggest that DHP (11) act in hormonal fashion (Abeles et al., 1972) and indirectly stimulate the biosynthesis of ethylene which then triggers seed germination (Logan and Stewart, 1991). The indirect role of an enzyme in converting a chemically unreactive "DHP (11)" to a reactive intermediate, possibly 38, seems to be a necessary, but not a sufficient condition. All these can be understood in terms of a plausible mechanism similar to that proposed by Adams and Yang (1979).
In considering stimulation of germination initiated by an alkylation process, it is important to recognize that DHP (11) contains as a reactive receptor site, the 4,5-epoxide. In general, epoxides have alkylating properties and can react with biological nucleophiles, in particular, thiol-containing amino acids of enzymes with essential thiol functions, e.g., L-cysteine and the enzymes phosphofructokinase and glycogen synthetase (Hall et al., 1977). In DHP (11) the epoxide function is not directly accessible due to steric hindrance of the associated medium ring structure. However, the epoxide provides increased reactivity, as well as regio- and stereospecificity of subsequent intramolecular cyclizations (Fischer, 1991). The epoxide is activated by bio-protonation and undergoes a Markovnikov-type transannular cyclization to give cation (38) as outlined in Scheme V.1. It is not unreasonable to hypothesize that the conditioned S. hermonthica seeds contain methionine (20) (active nucleophilic site is sulfur) which is also present in many plant tissues (Yang, 1974; Adams, 1977). It can be envisioned that, the cation (38) react with the sulfur group of methionine (20), a reaction mediated by pyridoxal phosphate. The positive charge imposed on the sulfur atom of 39 would create a strong leaving group, thus facilitating formation of Schiff base (22) via a concerted γ-elimination (Davis & Metzler, 1972) of the thiol moiety (41) along with α-hydrogen. This result in the formation cyclopropane adduct (24) which undergoes "chelotropic" elimination of ethylene as outlined in Scheme IV.1. It should be noted that conditioned Striga seeds have high oxygen (O2) tensions (Hsiao, 1981) necessary for the formation of hydrogen peroxide (H2O2) which reacts with ACC (16) leading to the formation of ethylene.

V.5. CONCLUSIONS. Our germination data demonstrate that DHP (11) possesses significant hormone-like activity in stimulating germination of conditioned S. hermonthica seeds. Thus in the future DHP (11) could prove very useful in control of S. hermonthica in agricultural situations where hormonal activity is desired. In addition, if DHP (11) mimics hormones in its mechanism of action, it may prove very useful in studies concerning the biochemistry of the actions of germination stimulants. Also, the carrier
system used may affect solubilization and activity of germination stimulants. It is hoped that the results obtained by MM analysis should be of great value in establishing the groundwork for the use of computer molecular modeling in explaining structure-activity relationships of agricultural compounds that exhibit *Striga* stimulatory activities. The goal of this tool in agriculture is to be predictive in the design of potent stimulatory substances.

\[
\text{trans,trans-farnesyl pyrophosphate (34)} \quad \alpha\text{-methylene-}\gamma\text{-lactone moiety (35)}
\]

\[
\text{Germacranolide (36)} \quad \text{iso-alantolactone (37)}
\]
Fig. V.1. Effect of dihydroparthenolide (DHP) (11) on germination of conditioned *Striga hermonthica* seeds. Data are means ± S. E. of twelve different experiments performed in triplicate (P = 0.05). Control is GR 24 (8) in methanol. Small vertical lines: standard error of mean value.
Table V.1. Calculated conformational energies (kcal/mol) of strigol (2), GR 24 (8) and DHP (11).

<table>
<thead>
<tr>
<th>Compound #</th>
<th>Me-15</th>
<th>Me-14</th>
<th>H-5</th>
<th>H-6</th>
<th>H-7</th>
<th>H-13</th>
<th>Energy</th>
<th>VDW*</th>
<th>DP* mom</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>β</td>
<td>β</td>
<td>α</td>
<td>β</td>
<td>α</td>
<td>β</td>
<td>25.40</td>
<td>4.78</td>
<td>5.41</td>
</tr>
<tr>
<td>11a</td>
<td>β</td>
<td>β</td>
<td>α</td>
<td>β</td>
<td>α</td>
<td>α</td>
<td>26.88</td>
<td>5.12</td>
<td>5.41</td>
</tr>
<tr>
<td>11b</td>
<td>β</td>
<td>α</td>
<td>α</td>
<td>β</td>
<td>α</td>
<td>β</td>
<td>104.04</td>
<td>1.84</td>
<td>2.81</td>
</tr>
</tbody>
</table>

VDW*, van der Waals volume. DP*, Dipole moment

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
Scheme V.1. Proposed mechanism for biosynthesis of ethylene from DHP (11). The substituted pyridinecarboxaldehyde stands for pyridoxal phosphate.
CHAPTER VI

ISOLATION OF DIHYDROELEPHANTOPIN,
ISODEOXYELEPHANTOPIN AND GRINDELIC ACID FROM THE
FAMILY ASTERACEAE
VI.1. INTRODUCTION

VI.1.1. Germacrane. Germacrane type sesquiterpenes are considered to be an important intermediates in the biosynthetic pathway towards guaiane-, eudesmane-, elemane- and other types of sesquiterpenes (Piet et al., 1995). Despite the fact that a large number of germacrane have been identified, the synthesis of these compounds is not well developed due to lack of suitable methods for construction of 10-membered rings with appropriate functional groups. The sesquiterpenoids are C-15 compounds formed of three isoprenoid units. Based on biogenetic assumptions, it is now generally accepted that all sesquiterpene lactones arise from a common precursor, farnesyl pyrophosphate, by various modes of cyclizations followed in many cases by skeletal rearrangements. In sesquiterpene lactones, the γ-lactone ring is capable of adopting two main conformations, S and A, as shown below. The A and the S conformations of the lactone ring differ by the sign of the torsion angle $\omega_4$. For the A conformation it is positive, and for the S conformation, it is negative. A trans-linked lactone ring has the S-conformation when the torsion angle $\omega_4 \leq 120^\circ$C, and the A-conformation when $\omega_4 \geq 120^\circ$C.

\[ \text{trans-lactone, S-type} \]
\[ \omega_4 \leq 120 ^\circ \text{C} \]

\[ \text{cis-lactone, S-type} \]

\[ \text{trans-lactone, A-type} \]
\[ \omega_4 \geq 120 ^\circ \text{C} \]

\[ \text{cis-lactone, A-type} \]
VI.1.2. *Elephantopus tomentosus* L. The genus *Elephantopus* (Compositae) is a member of the tribe Vernonieae, and is composed of approximately 32 species which are distributed throughout the tropical areas of the world. The chemistry of the genus *Elephantopus* is hitherto not well understood to clarify its phyletic position within the tribe Vernonieae; therefore, a survey of the chemistry of members of the genera composing this tribe is still needed. *E. tomentosus* L. (common names: tobacco-weed, devil's grandmother) is found in the United States and distributed from Eastern Texas through the Southeastern portions of the country. We now report the isolation and structure elucidation of dihydroelephantopin (10) as a major constituent of *E. tomentosus*.

VI.1.3. *Elephantopus carolinianus* Willd. The presence of desoxyelephantopin, a type of sesquiterpene lactone seems to be characteristic of this genus (Bohlmann *et al.*, 1984). From *E. carolinianus*, desoxyelephantopin and several other sesquiterpene lactones with marked anti-tumor activity have been isolated (Zhang *et al.*, 1986). Isodeoxyelephantopin (12) a minor constituent of this plant has been isolated previously from India *E. scaber* (Govindachari *et al.*, 1972), Sri Lanka *E. scaber* (De Silva *et al.*, 1982), West Virginia *E. carolinianus* and North Carolina *E. carolinianus* (Zhang *et al.*, 1986). 12 has shown significant cytotoxicity with ED$_{50} = 2.5$ µg/mL against the *in vitro* growth of KB tissue culture cells.

VI.1.4. *Grindelia maritima* L. *Grindelia* species are annual, biennial and perennial shrubs and herbs of the Asteraceae (tribe Astereae, subtribe Solidagininae). One of the main problems in the tribe Astereae is the delimitation of its subtribes (Jakupovic *et al.*, 1986); chemical characters therefore might prove helpful here. So far chemical investigations have shown that labdane diterpenes (which have the ent-labdane absolute configuration) and clerodane type are widespread in this subtribe (Bohlmann *et al.*, 1982; Adinolfi *et al*. 1988). The elucidation of the ecological roles of the resins in *Grindelia* spp. is only beginning. Resins are useful for a variety of industrial applications including
tackifiers, adhesives, and paper sizings; their derivatives are widely used in the production of synthetic polymers. Studies of resin biosynthesis, metabolism and transport will lead to significant taxonomic advances. The marked variation in both quantity and composition of resin produced by different populations make them attractive models for study of genetics and inheritance of resin production (Timmermann et al., 1987).

The North American *Grindelia maritima* L. (common name “gumweed”) is an annual herb and like the other members in the same genus, is characterized by the abundant production of resinous exudates which cover the surfaces of the leaves, the involucre of the flower heads and stems. The production and accumulation of these conspicuous hydrophobic, non-volatile resins appear to be associated with multicellular resin ducts which occur in the leaf mesophyll and stem cortex and sessile resin glands which occur in shallow pits on the surfaces of the leaves, stems, and involucre of the flower heads. Heavy resinous coatings may be a phytochemical and ecological adaptation to the arid and semi-arid environments to which these plants are exposed (Hoffmann et al., 1984).

VI.2. MATERIALS AND METHODS

VI.2.1. Isolation of Dihydroelephantopin (10). Aerial parts of *E. tomentosus* were collected at the flowery stage on October, 19, 1992 at Lee Forest Station, Washington Parish, Louisiana. A voucher specimen is on deposit in the Herbarium of LSU (Fischer collection 435. Air-dried leaf material (40 g) was ground in a Waring blender with 150 mL of dichloromethane (CH$_2$Cl$_2$). After filtration, the dichloromethane extract and washings were evaporated to afford a yellow-brown syrup which was chromatographed over a column of silica gel 60 (Merck). Elution was effected with hexane and EtOAc (gradient elution; increasing the volume of the more polar solvent, EtOAc). The medium polar fraction (EtOAc-hexane, 7:20 v/v) yielded 560 mg of
dihydroelephantopin (10) as a white crystalline solid \((C_{19}H_{22}O_7, M_r 362.38; \text{m.p.: } 266-268^\circ\text{C}; R_f 0.55, \text{EtOAc-hexane, 3:7}).\)

VI.2.2. **Isolation of Isodeoxyelephantopin (12).** The air-dried leaves (3.0 g) of *E. carolinianus* was extracted with dichloromethane at room temperature. The syrup (1.0 g) was column chromatographed on silica gel by elution with EtOAc-hexane (3:7). Fifty-three fractions of 4 mL were collected and examined by TLC. Fractions 25-27 yielded unstable crystals which were purified by prep. TLC [silica gel, \(C_6H_6-\text{CH}_2\text{Cl}_2-\text{Et}_2\text{O} (1:1:1)\)] and recrystallization from CHCl3-\text{Et}_2\text{O} afforded isodeoxyelephantopin (12) \([C_{19}H_{20}O_6, M^+ 344]\) (101 mg) as colorless needles: m.p. 161-162°C.

VI.2.3. **Isolation of Grindelic Acid (14).** *G. maritima* was collected by Dr. Fischer about 20 miles North of Santa Cruz, CA on Highway 1 between road and the coast on July 1, 1993. A voucher specimen is deposited in the Herbarium of LSU; collection *Fischer* 464. Ground dried aerial parts \{flowers (33 g), stems (46 g) and leaves (32 g)\} of the plant were exhaustively extracted using a number of different solvent systems (methanol, ethyl acetate, hexane). Dichloromethane was the most efficient solvent for the recovery of grindelic acid (14) \((C_{20}H_{32}O_3)\), although the drawback was that it also extracted pigments and chlorophyll. Evaporation of the solvent under reduced pressure yielded 5.0 g, 4.0 g and 3.0 g of the extractables from the flowers, leaves and stems, respectively. The dry extracts were chromatographed over silica gel packed in acetone \((\text{CH}_3)\text{CO})\). Fractions eluted with EtOAc-hexane (2:5 v/v) were rechromatographed to yield 1.6 g (32 %; calculated with respect to the crude extract), 1.0 g (25%) and 0.4 g (13%) of grindelic acid (14), respectively. Grindelic acid (14) was isolated as a yellow oil (TLC; \(R_f 0.67, \text{EtOAc-hexane, 7:20 v/v}\) and its spectral data \((\text{IR, } ^1\text{H, } ^{13}\text{C and GC/MS}) agreed with those reported in the literature \((\text{Brunn et al., 1981}).\)

VI.3. **RESULTS AND DISCUSSION.** The IR spectrum of dihydroelephantopin (10) contained absorptions typical of \(\alpha,\beta\)-unsaturated \(\gamma\)-lactone groups (endo: 1770;
exo: 1750 cm\(^{-1}\), double bonds (1660, 1640 cm\(^{-1}\)) and a sharp absorption at 1720 cm\(^{-1}\) for a saturated ester function. The \(^1\)H-NMR spectrum (CDCl\(_3\), 400 MHz) exhibited a broad singlet at 7.41 ppm for H-1; two doublets at 6.30 ppm and 5.80 ppm (J = 4 Hz) typical for the two methylene protons of the C11-C13 exocyclic double bond; a multiplet at 4.20 ppm for H-8; a complex multiplet between 2.60 ppm and 4.30 ppm for H-5 and H-6. In the high-field region of the spectrum a sharp singlet appeared at 1.32 ppm for the C-4 methyl group; the two side chain methyl groups (of the isobutyrate ester group) gave doublets at 0.87 ppm and 1.15 ppm (J = 5 Hz).

Our X-ray analysis data of dihydroelephantopin (10) tallied with those reported by Watson (1982). The data confirmed that 10 is a germacranolide-type sesquiterpene lactone containing a ten-membered ring which exhibits a chair-chair conformation in the solid state (torsion-angle sequence + - + - + - + - + -) which is found in most germacranolides. The C(4)-C(5) double bond of the germacradiene skeleton (34b) has been transformed into a trans epoxide function. The C(15) methyl group has been oxidized and forms an \(\alpha,\beta\)-unsaturated \(\gamma\)-lactone by closure at C(2) with the C(1)-C(10) double bond remaining undisturbed. The second \(\alpha,\beta\)-unsaturated lactone is trans-fused at C(6)-C(7) and exhibits an exocyclic double bond. The absolute configuration at C(6) is the same for all germacranolides, and the 6S configuration [i.e. H(6) is \(\beta\)] has been confirmed (Kupchan and Kelsey, 1967). The two five-membered rings in dihydroelephantopin (10) are flattened envelopes with C(6) and C(2) being the flaps. The C(13)C(11)C(12)O(12) and O(15)C(15)C(10)C(1) torsion angles are -05° and -178.3°, respectively.

The IR and NMR data of isodeoxyelephantopin (12) were very similar to those of dihydroelephantopin (10) and indicated the presence of an \(\alpha\)-methylene-\(\gamma\)-lactone [IR 1754 (\(\gamma\)-lactone) and 1635 (C=C) cm\(^{-1}\); \(^1\)H NMR: 5.67 ppm (1H, d, \(J_{7,13a} = 4.0\) Hz, H-13a) and 6.21 ppm (1H, d, \(J_{7,13b} = 4.0\) Hz, H-13b)] and a methacrylate ester group [IR: 1704 and 1636 cm\(^{-1}\); \(^1\)H NMR: 5.69 ppm and 6.16 ppm (each 1H, br s, H-18), and 1.94

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
(3H, dd, $J_{18,19a} = J_{18,19b} = 1.0$ Hz, H-19); MS: m/e 69 (base peak, [CH$_2$=CH(Me)CO]$^+$) and 258 (strong peak, [M-C$_4$H$_6$O$_2$]$^+$).

Unequivocal proof of the structure and stereochemistry of isodeoxyelephantopin (12) has been derived from single-crystal X-ray analysis of dimethylamine adduct (12b) by Zhang et al. (1986). However, their data departed significantly as compared to results from X-ray diffraction studies on related germacradienes (McPhail and Sim, 1973). With an $\alpha$-orientation of the oxygen substituent at C-2 in isodeoxyelephantopin (12), in contrast to the $\beta$-orientation at this center in dihydroelephantopin (10), formation of the corresponding $\gamma$-lactone ring incorporating C-15 requires that the latter also lies on the $\alpha$-face of the trans,trans-cyclodeca-1,5-diene ring, that is anti to the $\beta$-oriented C-14 methyl group.

Plant derived chemicals (pesticides) that deter herbivory are of great practical value (Elliger et al., 1976) and growing in demand, not only because of their derivation from a renewable source, but also because they are often highly specific, and most importantly, cause a minimum ecological disturbance. In the last few years considerable interest has been focused on the isolation and structure elucidation of grindelane diterpenoid acids, mainly due to insect feeding deterrent properties exhibited by some members of the genus *Grindelia* (Rose, 1980; Rose et al., 1981). To date, however, most successful discoveries of new insecticides from natural products have been from microbial transformations. For a long time, there existed no direct chemical synthesis of 3$\alpha$-hydroxygrindelic acid (14b), a potential starting material for a novel biorationally designed insecticides. Recently, Hoffmann and Punnpayak (1988) have successfully produced 14b from grindelic acid (14) through microbial chemical transformation involving *Aspergillus niger*. It is our hope that the naturally abundant grindelic acid (14) will be used to inhibit germination of *Striga* spp. seeds.

The dichloromethane extracts of the aerial parts of the previously uninvestigated *G. maritima* showed a definitive and elaborate quantitative variation of grindelic acid.
(14), the major constituent. The leaves contained the highest amount while the stems, the lowest. The observed quantitative differences in resin acid composition possibly represent differences in morphological characters of the aerial parts.

Our germination data (Figures VI.1-2) indicated that both dihydroelephantopin (10) and isodeoxyelephantopin (12) are promising germination stimulants for *Striga* spp. seeds. Grindelic acid (14) is a weak germination stimulant (Fig. VI.3.1-2). The carboxylic acid group is probably responsible for this behavior. This molecule might be used as a *S. hermonthica* seed germination inhibitor. Several derivatives of dihydroelephantopin (10), isodeoxyelephantopin (12), and grindelic acid (14) need to be synthesized and bioassayed in order to derive meaningful information concerning structure-activity relationships.

Isodeoxyelephantopin α-dimethylamine adduct (12b)
3α-hydroxygrindelic acid (14b)

Germacradiene skeleton (34b)
Fig. VI.1. Effect of dihydroelephantopin (10) and isodeoxeyelephantopin (12) on gemination of isolate of conditioned *Striga hermonthica* seeds occurring in Bida (A) location in Nigeria. Data are arc sine transformed relative percentage germination values expressed as means ± S. E. of twelve replicates (P = 0.05). Control is GR 24 (8) in methanol (fig. con'd).
Fig. VI.1. Effect of dihydroelephantopin (10) and isodeoxyelephantopin (12) on gemination of isolate of conditioned Striga hermonthica seeds occurring in Kano (B) location in Nigeria. Data are arcsine transformed relative percentage germination values expressed as means ± S. E. of twelve replicates (P = 0.05). Control is GR 24 (8) in methanol (fig. con'd).
Fig. VI.1. Effect of dihydroelephantopin (10) and isodeoxyelephantopin (11) on gemination of isolate of conditioned *Striga hermonthica* seeds collected in Abuja location in Nigeria during the 1992 (C) harvesting season. Data are arc sine transformed relative percentage germination values expressed as means ± S. E. of twelve replicates (P = 0.05). Control is GR 24 (8) in methanol (fig. con'd).
Fig. VI.1. Effect of dihydroelephantopin (10) and isodeoxyelephantopin (12) on germination of isolate of conditioned *Striga hermonthica* seeds collected in Abuja location in Nigeria during the 1993 (D) harvesting season. Data are arc sine transformed relative percentage germination values expressed as means ± S. E. of twelve replicates (P = 0.05). Control is GR 24 (8) in methanol.
Fig. VI.2. Effect of dihydroelephantopin (10) and isodeoxyelephantopin (12) on germination of isolate of conditioned *Striga aspera* seeds collected in Kano location (E) in Nigeria during the 1993 harvesting season. Data are arc sine transformed relative percentage germination values expressed as means ± S.E. of twelve replicates (P = 0.05). Control is GR 24 (8) in methanol.
Fig. VI.3.1. Effect of grindelic acid (14) on germination of conditioned *Striga hermonthica* seeds occurring in Bida (A) and Kano (B) locations in Nigeria. Data are arc sine transformed relative percentage germination expressed as means ± S. E. of twelve replicates (P = 0.05). Control is GR 24 (8) dissolved in methanol (fig. con'd).
Fig. VI.3.1. Effect of grindelic acid (14) on germination of conditioned *Striga hermonthica* seeds collected in Abuja location in Nigeria during the 1992 (C) and 1993 (D) harvesting seasons. Data are arc sine transformed relative percentage germination expressed as means ± S. E. of twelve replicates (P = 0.05). Control is GR 24 (8) dissolved in methanol.
Fig. VI.3.2. Effect of grindelic acid (14) on germination of isolate of conditioned *Striga aspera* seeds collected in Kano location (E) in Nigeria during the 1993 harvesting season. Data are arc sine transformed relative percentage germination values expressed as means ± S. E. of twelve replicates (P = 0.05). Control is GR 24 (8) in methanol.
CHAPTER VII

RESPONSE OF TWO DIFFERENT ISOLATES OF *STRIGA HERMONTHICA* SEEDS TO VARYING CONCENTRATIONS OF STRIGOL ANALOG GR 7
VII.1. INTRODUCTION. Populations of Striga species from different geographical areas and different hosts have been reported to exhibit marked differences in virulence. Striga hermonthica (Del.) Benth. has a high level of genetic diversity (Bharathalakshmi et al., 1990), even in comparison with other species that share its life-history features. There are many reports of inter-provenance differences in virulence for S. hermonthica parasitizing a given cereal cultivar host (King and Zummo, 1977). Several lines of evidence indicate that the control of S. hermonthica presents a serious problem, because economically effective means of control are not yet available for small-scale farmers in developing countries. The high application of nitrogen fertilizer needed to bring about a significant reduction in Striga infestation is prohibitively expensive for small-scale farming. Besides, the paradoxical effect (Pieterse, 1991; Pieterse and Verkleij, 1991) is yet to be fully understood. The introduction of synthetic germination stimulants to deplete Striga seed reserves in the soil is an intriguing possibility (Egley and Dale, 1970). Various reports have indicated that the strigol analogues, such as GR 7 (9) could induce germination of several Striga species and strains (Hassanali, 1983; Ibrahim et al., 1985). Johnson et al. (1981) described the preparation of GR 7 (9) and GR 24 (8). Their results indicate that the former can readily be synthesized in large quantities from the so-called "Corey's lactone" following the standard coupling procedure. "Corey's lactone" is commercially available (even in optically pure forms), which makes GR 7 (9) an attractive compound for germination stimulation of seeds of Striga species. The objective of this work was to assess the effects of different concentrations of GR 7 (9) on germination of isolates of S. hermonthica seed lots collected in Bida and Abuja locations in Nigeria (Experimental details as described in Materials and Methods of Chapter IV).
VII.2. RESULTS

VII.2.1. Effect of Several Concentrations of GR 7 (9) on the Induction of Germination of Conditioned *Striga hermonthica* Seeds. In the laboratory, at 28°C during the 24h period, germination of conditioned *S. hermonthica* seeds did not occur in either 0.1 % methanol or sterile double-distilled deionized water, the negative controls. Aqueous solutions of GR 7 (9) and the positive control, GR 24 (8) induced germination. Activity of the solutions as indicated by percentage germination was dependent on concentration of GR 7 (9) and the *S. hermonthica* isolate under investigation (Figures VII.1A-C). There was not an obvious relationship between concentration and germination of the parasite seeds. At $10^{-3}$ M, GR 7 (9) was less stimulatory on germination of both isolates. Surprisingly, $10^{-19}$ M solution of GR 7 (9) showed higher germination rate than the positive control on both isolates of *S. hermonthica*. The isolate of *S. hermonthica* collected from Bida showed the highest germination responses which were not reproduced by either of the isolate from Abuja location. Moreover, this isolate gave 52-81% germination, while those collected from Abuja during the 1992 and 1993 harvesting seasons resulted in 21-32% and 18-42% germination, respectively. The isolate from Abuja (1993) gave higher germination rates than GR 24 (8) in all concentrations except at $10^{-6}$, $10^{-8}$, $10^{-14}$ and $10^{-15}$ M. A low “double maximum” of germination at 33% was noted for the isolate from Abuja (1992) which occurred at $10^{-7}$ and $10^{-13}$ M.

VII.3. DISCUSSION

VII.3.1. Selection Criteria for Germination Stimulants. To date, GR-compounds (GR is referring to Gerry Rosebery, one of the members of Johnson's group), especially GR 24 (8) and GR 7 (9) are the most widely field evaluated multiring strigol analogues. These compounds, developed to control *Striga* spp., meet the several criteria for germination stimulants for field use: (1) activity at low concentration; (2) reasonable water solubility; (3) environmental safety; and (4) ability to promote
germination in a range of species (Bradow, 1986; Egley, 1980). Specifically, GR 24 (8) (mol. wt. 298) has been shown to stimulate *Alectra vogelii* Benth. (Visser and Johnson, 1982) and *S. hermonthica* but not *S. gesnerioides* (Willd.) Vatke (Okonkwo and Okafor, 1988). The other strigol analogue, GR 7 (9) (mol. wt. 248), has been shown to exhibit a high germination activity for *S. hermonthica* as well as *S. asiatica* (L.) Kuntze and *Orobanche ramosa* L. both in the field (Pavlista and Worsham, 1979; Johnson *et al*., 1976) and in the laboratory (Johnson and Hassanali, 1981a,b). However, it has been shown that GR 7 (9) was more active *in vitro* (Johnson *et al*., 1976) than when soil applied (Babiker and Hamdoun, 1979; Stevens and Eplee, 1979). GR 7 (9) shows adequate persistence (6-8 days) in acidic soils (pH 5.0-6.3), but residual activity is short (1-3 days) in alkaline soils (Babiker, 1988). This decreased activity in soils was attributed to the breakdown of the chemical (Stevens and Eplee, 1979).

**VII.3.2. Factors which affect Germinability of *Striga hermonthica* Seeds.**

Despite the enormous seed production by weeds which help them maintain their populations (Chandler *et al*., 1977), the number of seeds reaching the soil bank is limited by various environmental factors. A proportion of the seeds produced by *Striga* spp. plants is carried a considerable distance away by activities of man. Losses due to seed eating animals and birds, and destructive microbes also affect the number of germinable seeds entering the seed pool.

Factors such as soil type, soil moisture, soil fertility, temperature, light, rainfall pattern during the season, crop planting date, and synchronization of the host growing cycle with the growing season can have a significant effect on germinability of *Striga* seeds in the laboratory. These arguments find further support from Babiker *et al.* (1988) who have shown that the germination of *S. hermonthica* seeds in response to GR-compounds was influenced by soil moisture and soil pH. Ogbom (1972) observed that *Striga* spp. seeds thrives on intermittent dry conditions and is suppressed by continuous soil moisture. Excessive soil moisture decreases oxygen level in the soil and induces
In anaerobic conditions (Kimmerer and Kozlowski, 1982). Oxygen may be vital for an oxidative process, which leads to removal of an inhibitor(s), or attainment of critical levels of required factors or biochemical stages needed for the stimulant to be effective. In addition, anaerobic conditions may increase accumulation of ethanol and acetaldehyde, which affect seed dormancy and germination (Davis, 1980; Taylorson and Hendricks, 1981). The inhibitory effects of these compounds are pronounced at low oxygen levels (Holm, 1972). There is thus good reason to believe that all or a combination of these factors affect the survival of germinable seed lots of the parasite recovered from the soil in Bida and Abuja locations.

A noteworthy aspect of the present investigation is the observation that the isolates of *S. hermonthica* occurring in Bida and Abuja locations exhibited marked differences in germination responses to various concentrations of GR 7 (9) (Figures VII.1A-C). Generalizations about the influence of the various concentrations on germination should be made with caution. The germination data could be rationalized from several perspectives. The adverse effect that application of 10^{-3} M GR 7 (9) had on responsiveness of all isolates of *S. hermonthica* is in line with the findings of Fischer et al. (1990) for *Striga asiatica*. The adverse effect might be, as suggested by Pavlista et al. (1979), due to the interference of GR 7 (9) with leaching of an inhibitor from the seed. However, interactions between GR 7 (9), the inhibitor and/or other components of the system cannot be dismissed. Prior to germination, *Striga* spp. seed requires a period of conditioning, the optimum time being temperature dependent (Logan and Stewart, 1992). Previous studies by Jackson et al. (1991) have shown that *S. hermonthica*, and probably *S. aspera* and *S. asiatica*, require the joint action of ethylene (produced by the seed itself) and strigol-related substances before they will germinate. In our experiment, we employed a 10-day conditioning period and 24 h incubation with the test solutions (GR 7 (9) and GR 24 (8)). We cannot rule out the possibility that these experimental conditions
might not have been adequate for all the isolates of *S. hermonthica* occurring in Bida and Abuja locations.

Isolate of *S. hermonthica* from Bida gave the highest response to all concentrations of GR 7 (9). This isolate might be the most virulent parasite in the field. The very low percentages of germinable seeds particularly those collected in Abuja in 1992 was evident. This may be due to the loss of some seeds via germination before the collection of soil samples and also to the presence of non-germinable seeds from the preceding year. In addition, some of the seeds which did not germinate were probably under induced or enforced dormancy (viable but non-germinable in the laboratory). This is in agreement with the observation made by Hintikka (1988) that the seeds of many plant species enter dormancy when buried in the soil or humus. However, factors that cause the onset of dormancy remain to be fully understood.

**VII.4. CONCLUSION.** Isolates of conditioned *Striga hermonthica* occurring in Bida and Abuja locations in Nigeria exhibited different germination responses to GR 7 (9) under laboratory conditions. It is possible that the different isolates have different seed physiologic specialization in mechanism of dormancy. Using GR 7 (9) and other potentially promising germination stimulants, further experimental work is needed, both in the laboratory and in the field.
Fig. VII.1. Effect of GR 7 (9) on germination of conditioned *Striga hermonthica* seeds collected in Abuja location during the 1992 (A) and 1993 (B) harvesting seasons. Data are arcsine transformed percentage germination values expressed as means ± S.E. of twelve replicates (P = 0.05). S.E. shown only when larger than the symbol. Control is GR 24 (8) in methanol (fig. con'd).
Fig. VII.1. Effect of GR 7 (9) on germination of isolate of conditioned *Striga hermonthica* seeds collected in Bida (C) location in Nigeria. Data are arc sine transformed relative percentage germination values expressed as means ± SE of twelve replicates (P = 0.05). SE shown only when larger than the symbol. Control is GR 24 (8) in methanol.
CHAPTER VIII

CADINANES ISOLATED FROM HETEROTHECA SUBAXILLARIS LAM.*

*This chapter contains some materials which have been reprinted with permission from Spectroscopy Letters.

135
VIII.1. INTRODUCTION. The polymorphic *Heterotheca subaxillaris* (Lam.) Britton & Rusby (family Asteraceae; tribe Astereae), commonly referred to as camphorweed, is widespread, spanning the coastal plain of the Southern and Eastern United States. Although the chemistry of many Asteraceae has been investigated, members of the tribe Astereae have received only limited attention (Lincoln and Lawrence, 1984). These taxa appear to be characterized by the absence of sesquiterpene lactones or alkaloids, but commonly accumulate mono-, sesqui- and diterpenoids. The five species of the North American genus *Heterotheca*, which have so far been investigated chemically are rich in cadinanes which exhibit diverse biological activities with remarkable dependence on minor structural and stereochemical changes (Kalsi and Talwar, 1981; Borg-Karlson and Norin, 1981). Cadinanes, which are common in higher plants, have been further divided into four subclasses based on the nature of the ring fusion and the orientation of the isopropyl group at C(10) (Bordoloi et al., 1989). Due to complex stereochemistry of these compounds, many of the previous structural and configurational assignments appear to be uncertain (Borg-Karlson and Norin, 1981). It is also evident that the present classification of cadinane-type compounds needs a revision of nomenclature (Vlahov et al., 1967).

$^{13}$C NMR represents an important tool for structure determination, since $^{13}$C chemical shifts are far more sensitive to their chemical environment than proton shifts, providing valuable structural information (Bax et al., 1986). The normal and DEPT-edited experiment (Doddrell et al., 1982) can be used to partially assign $^{13}$C spectra in terms of the number of attached protons. Furthermore, the recent development of two-dimensional NMR spectroscopy has provided a number of new NMR assignment techniques which are useful in the area of natural products chemistry (Derome, 1989; Shoolery, 1984). In particular, the direct heteronuclear ($^{1}$H-$^{13}$C) chemical shift-correlated spectra allow simultaneous determination of $^{1}$H and $^{13}$C chemical shifts for directly bonded $^{13}$C$^{1}$H$_{n}$ units (Bax and Morris, 1981).
As part of an ongoing study of 2-hydroxy-8α-angeloyloxycalamenene (13) and 2-hydroxy-8α-hydroxycalamenene (42) as germination stimulants for *Striga* spp. seeds, it was deemed important to report their complete $^{13}$C NMR assignments, information which is invaluable for the structure elucidation of other terpenoids in general and calamenene-type compounds in particular. The calamenenes are A-ring-aromatized cadinanes with a methyl group at the C-7 position (Bordoloi *et al.*, 1989). It is of some medicinal interest to note that the skeleton of 2-hydroxy-8α-angeloyloxycalamenene (13) and 2-hydroxy-8α-hydroxycalamenene (42) is the same as that of the spermicidal gossypol (Davila-Huerta *et al.*, 1995). Unambiguous assignments for all skeletal carbon resonances of both compounds were made primarily by the application of 2D $^{13}$C-$^1$H heteronuclear correlated spectroscopy (HETCOR) (Bax and Morris, 1981) and long-range heteronuclear correlation experiments (COLOC) (Kessler *et al.*, 1984).

VIII.2. EXPERIMENTAL

VIII.2.1. Plant Materials. The aerial parts of *H. subaxillaris* were collected by N. H. Fischer, H. D. Fischer and L. Quijano on February 2, 1989, in Jefferson Parish along the beach at Grand Isle, Louisiana, U.S.A. (Voucher No. Fischer 375; voucher deposited at the Louisiana State University Herbarium). IR spectra were recorded on a Perkin-Elmer 1760x FT-IR spectrometer as a film on KBr plate using CHCl$_3$ solution. Mass spectra were run on a Hewlett-Packard 5971 A GC/MS spectrometer. Comparison by TLC (Fischer, 1991) (EtAOc-hexane, 2:5), GC/MS and $^1$H NMR of the crude extract of stems and leaves showed that they contained the same compounds with minor quantitative differences.

VIII.2.2. Extraction and Isolation. Powdered leaves (502 g) were extracted with CH$_2$Cl$_2$ (6 x 900 mL) at room temperature for 72 h. Evaporation of the combined CH$_2$Cl$_2$ extracts under reduced pressure gave 5.6 g of crude extract which was chromatographed by VLC (Pelletier *et al.*, 1986) using 100 g silica gel (MN Kieselgel G) and ethyl acetate-hexane as eluent. The percentage of EtOAc in hexane was gradually
increased \((\text{EtOAc}:\text{hexane (v/v)}: 0:1; 1:9; 1:4; 3:7; 1:1; 7:3; 9:1; 1:0, 50 \text{ mL each})\). Forty fractions of 10 mL each were collected and monitored by TLC performed on precoated MN Sil-G 25 UV \(_{254}\) plates having a layer thickness of 0.25 mm. Fraction 12 provided 14 mg of 2-hydroxy-8\(\alpha\)-angeloyloxycalamene (1) \([R_f 0.86 (\text{SiO}_2, \text{EtAOc-hexane}, 2:5)]\) as a yellow oil. The medium polar fractions 20-25 eluted with 25\% EtAOc-hexane were combined and evaporated to afford 120 mg of 2-hydroxy-8\(\alpha\)-hydroxycalamene (42) \([R_f 0.45 (\text{SiO}_2, \text{EtOAc-hexane}, 2:5)]\) as a yellow oil.

**VIII.2.3. NMR.** One and two-dimensional NMR spectra were obtained in a Bruker AMX 400 NMR spectrometer at room temperature. Samples of 6 mg of 1 or 2 were dissolved in 0.7 mL of CDCl\(_3\) (99.8\% isotopically pure in deuterium, from Aldrich Chemical Company Inc., Milwaukee, U.S.A) in 5 mm diameter NMR tubes. The \(^1\text{H}\) and \(^{13}\text{C}\) chemical shifts are reported in \(\delta\)-values (ppm) and referenced to the signal of CDCl\(_3\) at 7.26 and 77.00 ppm, respectively. To make unambiguous assignments of carbon signals additional \(^1\text{H}-^1\text{H}\) COSY (Aue et al., 1976), NOESY (Bax and Morris, 1981), DEPT (Doddrell et al., 1982), \(^{13}\text{C}-^1\text{H}\) correlation (HETCOR) (Bax and Morris, 1981) and COLOC (Kessler et al., 1984) experiments were performed. The 2D experiments were acquired and processed with the software provided by Bruker on AMX 400. Typical acquisition time and processing conditions for COSY and NOESY experiments were: relaxation delay of 1 and 2 seconds, 512 \(t_1\) increments; 1024 to 2048 \(t_2\) points; sweep width of 2 ppm. Sine bell squared and shifted \((\pi/4, \pi/6\) and \(\pi/8)\) apodization functions were used for processing. The mixing time in NOESY experiments, generally set at 1.2-1.5 seconds, was also varied between 0.8 and 2 seconds, without substantial change in the results. For \(^1\text{H}-^{13}\text{C}\) \((^{13}\text{C} \text{ detected})\) correlations, the same relaxation delays were used, 256 to 512 \(t_1\) increments, 1024 to 2048 \(t_2\) points, the sweep width being 60 ppm for \(^{13}\text{C}\). Lorentz and Gaussian deconvolution were generally used in the processing. The number of scans was set for an overall acquisition time of about 12 to 16 hours. HETCOR was carried out at a \(^{13}\text{C}\)
frequency of 100.62 MHz using 4K data points in the \( t_2 \) dimension and 256 increments on \( t_1 \). A Gaussian window function was applied to the \( t_2 \) dimension and a sinebell window function was applied to the \( t_1 \) dimension. Zero filling to 1K was also applied in the \(^1\)H dimension. Three-bond long-range \(^1\)H-\(^{13}\)C shift correlations (COLOC) experiment were performed using a pulse sequence reported by Kessler \( et \) \( al. \) (1984).

The data were acquired in 12 hours using 256 experiments, each with a block size of 4K. The following parameters were used: \( D_1 = 2 \) sec, \( D_f = 3 \) msec, \( D_3 = 0.071 \) sec, \( D_4 = 0.036 \) sec. The \( D_3 \) and \( D_4 \) values were computed using the observed three-bond coupling constant, \( ^3J_{CH} = 7 \)Hz. The data were processed using sinebell multiplication in both dimensions (SSB1 = SSB2 = 0) and Gaussian multiplication in the second dimension (WDW2 = G; LB2 = 2.0) before Fourier transformation. The contour plot was plotted at the 256 K level.

**VIII.3. RESULTS AND DISCUSSION.** The \(^1\)H NMR spectrum of the crude dichloromethane extract of \( H. \) \( subaxillaris \) showed absorptions both in the aromatic region as well as aliphatic methyl signals. From fractions of the medium polarity fractions, the cadinanes, 2-hydroxy-8\( \alpha \)-angeloyloxy calamene (13) and 2-hydroxy-8\( \alpha \)-hydroxycalamene (42) were isolated. Both compounds had previously been isolated from \( H. \) \( subaxillaris \) of an unspecified collection site and their structures were determined by spectroscopic methods (Bohlmann and Zdero, 1979).

**VIII.3.1. \(^{13}\)C NMR Spectral Assignments in 2-hydroxy-8\( \alpha \)-angeloyloxy calamene (13).** The spectral data (\(^1\)H NMR, IR, MS) of 2-hydroxy-8\( \alpha \)-angeloyloxy calamene (13) were in full agreement with the reported values (Bohlmann and Zdero, 1979). Only the homonuclear coupling constants in the \(^1\)H NMR spectrum showed marginal differences. The \(^{13}\)C NMR spectrum of 13 indicated the presence of 20 carbon signals in the molecule. Its relative stereochemistry was determined from NOESY (Bax and Morris, 1981) experiment and proton coupling data which allowed the unambiguous assignment of carbon and proton resonances (Table
VIII.1). The DEPT spectrum of 13 (Fig. VIII.1) exhibited six methyls, one methylene and seven methine signals, while the remaining 6 signals in the broad-band spectrum were due to the quaternary carbon atoms. The methyl protons were assigned by analyses of COSY (Aue et al., 1976), NOESY (Bax and Morris, 1981), and 2D one-bond heteronuclear correlation (HETCOR) (Bax and Morris, 1981) contour diagrams shown in Figures VIII.2, VIII.3, and VIII.4, respectively. The aromatic methine protons H-1 and H-4 at 6.58 and 6.92 ppm showed cross peaks with carbon resonances at 113.6 and 130.3 ppm, respectively (Fig. VIII.4.1). The upfield region of the HETCOR spectrum (Fig. VIII.4.2) showed cross peaks of the methyl protons H-12, H-13, H-14, H-15, H-4' and H-5' at 17.2, 21.1, 17.3, 15.6, 15.5, and 20.5 ppm, respectively. The signal at 72.3 ppm was due to the oxygen-bearing carbon, C-8 (Silverstein et al., 1991). The $^{13}$C NMR resonances of one methylene carbon (C-9) and the remaining 5 methine carbons were similarly assigned from the correlation diagram (Fig. VIII.4.1) and are depicted in Table VIII.1. The only difficulty in assignment of the $^{13}$C NMR resonances came from the non-protonated carbons, C-2, C-3, C-5, C-6, C-1', and C-2' which do not have cross peaks in the HETCOR diagram. To unambiguously assign these $^{13}$C NMR signals, a three-bond long-range heteronuclear correlation experiment (COLOC) was carried out. Figure VIII.5 shows the non-protonated carbons at the downfield region of the COLOC contour plot for 2-hydroxy-8α-angeloyloxy-calamenene (13). The carbonyl resonance at 168.1 ppm showing long-range correlation with the H-5' was assigned to C-1'. Based on the correlation with the H-5' proton signal, the carbon resonance at 128.2 ppm could be assigned to C-2'. Three carbon resonances at 130.3 (C-4), 152.0, and 121.7 ppm showed correlation with H-15, indicating either a two-bond or three-bond coupling to H-15. The carbon resonances at 121.7 and 152.0 ppm were tentatively assigned to C-3 and C-2, respectively. The carbon resonance at 152.0 ppm showed correlation peaks with H-1, H-4 and H-15 could be unambiguously assigned to C-2, whereas the carbon at 121.7 ppm was assigned to C-3, values in accord with chemical
shift considerations. Three correlation peaks were observed between the H-4 proton signal at 6.92 ppm and carbon resonances at 130.4 (C-4), 152.0 (C-2) and 138.8 ppm. The carbon signal at 138.8 ppm showed a three-bond correlation with C-4 and was unambiguously assigned to C-6. The remaining \(^{13}\)C signal at 130.0 ppm showed a three-bond correlation peak with the H-1 signal at 6.58 ppm and was therefore assigned to C-5.

VIII.3.2. \(^{13}\)C NMR Spectral Assignments in 2-hydroxy-8\(\alpha\)-hydroxycalamene (42). The structure of 2-hydroxy-8\(\alpha\)-hydroxycalamene (42) was illustrated to be a cadinane sesquiterpenoid with a secondary hydroxyl group at C-8. Its spectral data resembled those of 13 occurring in the same plant. The IR spectrum of 42 showed the presence of hydroxyl and double bond (C=C) absorptions at 3500 and 1650 cm\(^{-1}\), respectively. The high resolution \(^1\)H NMR spectrum provided considerable information (Table VIII.2) and the chemical shift values were in close agreement (differences of < 0.01 ppm) with the literature data (Bohlmann and Zdero, 1979). There were 1H singlets at 6.95 and 6.61 ppm indicating the aromatic hydrogens (H-4 and H-1, respectively) in a para relationship. A singlet for an aromatic methyl group appeared at 2.20 ppm and an OH group signal at 6.15 ppm. The remaining two positions of the benzene ring are occupied by benzylic MeCH< and Me\(_2\)CHCH< groups (the underlined protons being vicinal to the phenyl ring).

The 2D \(^1\)H-\(^1\)H COSY and NOESY experiments revealed and clarified the presence and connectivities of each of the three partial segments, A - C:
One oximethinic proton (H-8) located downfield at 4.12 ppm correlated with both methylene protons at C-9 carbon and a secondary methyl (H-14) in segment A. The C-4 proton (H-4) correlated with a tertiary methyl (H-15) in segment B and H-1 and in segment A. Two secondary isopropyl methyl groups (H-12 and H-13) were correlated with the methine proton (H-11) in segment C and with H-14 methyl group in segment A. A pair of 3H doublets \( J = 6.8 \) Hz at 0.66 and 0.98 ppm, which were scalar-coupled to a 1H multiplet at 2.20 ppm in COSY spectrum (Figure VIII.6) confirmed the presence of an isopropyl moiety (segment C). Moreover, the EI-mass spectrum established a molecular weight of 234 \([\text{M}]^+\) (corresponding to \( \text{C}_{15}\text{H}_{22}\text{O}_2 \)) with major fragment ions resulting from loss of isopropyl group.

The stereochemistry of 2-hydroxy-8\( \alpha \)-hydroxycalamenene (42) was deduced by the NOESY experiment. Several significant NOEs observed were not in agreement with the previously assigned stereostructures of 2-hydroxy-8\( \alpha \)-angeloyloxy calamene (13) and 2-hydroxy-8\( \alpha \)-hydroxycalamenene (42) (Bohmann and Zdero, 1979). As noticeable in Figures VIII.3 and VIII.7, cross peaks between H-12 and H-11, H-13 and H-14, between H-14 and H-11 strongly suggested that the C-7 methyl group and the C-10 isopropyl group in both 13 and 42 are cis-oriented. Chemical shifts correlations, integrals and determination of the relevant coupling constants (e.g. \( J_{9,10} \) and \( J_{7,8} \)) further supported the relative configurations at C-7, C-8 and C-10.

The \( ^{13}\text{C} \) NMR spectrum of 2-hydroxy-8\( \alpha \)-hydroxycalamenene (42) showed signals of fifteen carbon atoms, four of them being quaternary. The protonated carbon signals were assigned by the polarization transfer experiment (DEPT) (Figure VIII.8) which indicated the presence of four methyl groups, one methylene and six methines (one directly bonded to an oxygen function at 70.7 ppm). Two sets of aromatic carbon signals arose at \( d 113.6, 130.3 \) and were attributed to \(-\text{C}=\text{CH}\) and at \( d 121.8, 130.6, 138.0, 152.2 \) due to quaternary aromatic carbons. Two-dimensional \( ^{13}\text{C}-^1\text{H} \) COSY spectral data (Figures VIII.9.1 and VIII.9.2) was employed in the assignments of chemical shifts of
protonated carbons in the $^{13}$C NMR spectrum. Quaternary carbons (C-2, C-3, C-5 and C-6) were assigned by comparison with the spectral data for 13 and for similar skeletal structures (Davila-Huerta et al., 1995).

Because 2-hydroxy-8α-angeloyloxyccalamene (13) is the major constituent of $H$. subaxillaris, various concentrations were assessed for germination of conditioned *Striga* seeds. The results are depicted in Figures VIII.9.3-4. Isolates of *S. hermonthica* occurring in Bida (Fig. VIII.9.3A) and *S. aspera* occurring in Kano (Fig. VIII.9.4E) showed higher germination responses to 13 than the control (GR 24 (8)). More work is needed to establish the mechanism of germination/inhibition of *Striga* seeds by 2-hydroxy-8α-angeloyloxyccalamene (13). The presence of two antagonistic functional groups, the OH (inhibitor of germination) as well as the angelate ester group (a Michael receptor site for methionine (20) which can lead to formation of ethylene) makes this molecule an interesting model for future studies.
2-hydroxy-8α-angeloyloxy calamene (13)

2-hydroxy-8α-hydroxycalamene (42)
Table VIII. 1. $^{13}$C-$^1$H correlation of 2-hydroxy-8α-angeloyloxy calamene (13) (400 MHz, CDCl$_3$)

<table>
<thead>
<tr>
<th>$\delta^{13}$C</th>
<th>Mult.$^a$</th>
<th>$\delta_{\text{attached}}$H</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>113.6</td>
<td>CH</td>
<td>6.58 s</td>
<td>1</td>
</tr>
<tr>
<td>152.0</td>
<td>C$^d$</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>121.7</td>
<td>C$^d$</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>130.4</td>
<td>CH</td>
<td>6.92 s(br)</td>
<td>4</td>
</tr>
<tr>
<td>130.0</td>
<td>C$^d$</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>138.8</td>
<td>C$^d$</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>36.1</td>
<td>CH</td>
<td>3.05 dq</td>
<td>7</td>
</tr>
<tr>
<td>72.3</td>
<td>CH</td>
<td>5.37 ddd</td>
<td>8</td>
</tr>
<tr>
<td>25.2</td>
<td>CH$_2$</td>
<td>1.85 ddd; 2.05 ddd</td>
<td>9</td>
</tr>
<tr>
<td>40.8</td>
<td>CH</td>
<td>2.80 ddd</td>
<td>10</td>
</tr>
<tr>
<td>32.9</td>
<td>CH</td>
<td>2.10 dqq</td>
<td>11</td>
</tr>
<tr>
<td>17.2$^b$</td>
<td>CH$_3$</td>
<td>0.99 d</td>
<td>12</td>
</tr>
<tr>
<td>21.1$^c$</td>
<td>CH$_3$</td>
<td>0.75 d</td>
<td>13</td>
</tr>
<tr>
<td>17.3</td>
<td>CH$_3$</td>
<td>1.21 d</td>
<td>14</td>
</tr>
<tr>
<td>15.6</td>
<td>CH$_3$</td>
<td>2.10 s(br)</td>
<td>15</td>
</tr>
<tr>
<td>168.1</td>
<td>C$^d$</td>
<td>-</td>
<td>1'</td>
</tr>
<tr>
<td>128.2</td>
<td>C$^d$</td>
<td>-</td>
<td>2'</td>
</tr>
<tr>
<td>137.6</td>
<td>CH</td>
<td>6.02</td>
<td>3'</td>
</tr>
<tr>
<td>15.5</td>
<td>CH$_3$</td>
<td>1.89</td>
<td>4'</td>
</tr>
<tr>
<td>20.5</td>
<td>CH$_3$</td>
<td>1.85</td>
<td>5'</td>
</tr>
</tbody>
</table>

J(Hz): 7α, 8β, = 4.4; 7α, 14 = 6.8; 8β, 9β = 8.5; 9α, 9β = 14; 9α; 10α = 9β, 10α = 10α, 11 = 6; 11, 12 = 11, 13 = 6.8.

$^a$ Carbon multiplicities determined through DEPT experiments.

$^b,^c$ Assignments may be interchanged in each vertical column.

$^d$ Assignments based on COLOC experiment.
Table VIII.2. $^1$H- and $^{13}$C NMR spectral data (δ) for 2-hydroxy-8α-hydroxycalamene (42) (400 MHz, CDCl$_3$)

<table>
<thead>
<tr>
<th>$\delta^{13}$C</th>
<th>Mult.$^a$</th>
<th>$\delta$attachedH</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>113.6</td>
<td>CH</td>
<td>6.61 s</td>
<td>1</td>
</tr>
<tr>
<td>152.2</td>
<td>C$^b$</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>121.8</td>
<td>C$^b$</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>130.3</td>
<td>CH</td>
<td>6.95 s(br)</td>
<td>4</td>
</tr>
<tr>
<td>130.6</td>
<td>C$^b$</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>138.0</td>
<td>C$^b$</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>37.7</td>
<td>CH</td>
<td>2.84 dq</td>
<td>7</td>
</tr>
<tr>
<td>70.7</td>
<td>CH</td>
<td>4.12 ddd</td>
<td>8</td>
</tr>
<tr>
<td>28.9</td>
<td>CH$_2$</td>
<td>1.50 ddd; 1.95 ddd</td>
<td>9</td>
</tr>
<tr>
<td>39.4</td>
<td>CH</td>
<td>2.85 ddd</td>
<td>10</td>
</tr>
<tr>
<td>33.0</td>
<td>CH</td>
<td>2.20 dqq</td>
<td>11</td>
</tr>
<tr>
<td>16.6$^c$</td>
<td>CH$_3$</td>
<td>0.98 d</td>
<td>12</td>
</tr>
<tr>
<td>20.8$^c$</td>
<td>CH$_3$</td>
<td>0.66 d</td>
<td>13</td>
</tr>
<tr>
<td>16.6</td>
<td>CH$_3$</td>
<td>1.29 d</td>
<td>14</td>
</tr>
<tr>
<td>15.5</td>
<td>CH$_3$</td>
<td>2.20 s(br)</td>
<td>15</td>
</tr>
</tbody>
</table>

$^a$ Carbon multiplicities determined through DEPT experiments.
$^b$ These carbon number assignments were made by comparison with assignments for 13 and related compounds (Davila-Huerta, et al., 1995).
$^c$ Assignments may be interchanged in each vertical column.

J (Hz): 7α, 8β, = 3.5; 7α, 14 = 7; 8β, 9β = 3; 9β, 9α = 13.5; 9α, 10α = 9β; 10α = 7; 10α, 11 = 6.
Figure VIII.1. DEPT 90, 135 and BB $^{13}$C NMR spectra of 2-hydroxy-8$\alpha$-angeloyloxycalamenene (13).
Figure VIII.2. 2D $^1$H NMR COSY spectrum of 2-hydroxy-8$\alpha$-angeloyloxy-calamenene (13).
Figure VIII.3. 2D $^1$H NMR NOESY spectrum of 2-hydroxy-8α-angeloyloxy-calamenene (13).
Figure VIII.4.1. 2D $^{13}$C-$^1$H HETCOR spectrum of 2-hydroxy-8α-angeloyloxyxcalamene (13).
Figure VIII.4.2. Upfield region of HETCOR spectrum of 2-hydroxy-8α-angeloyloxycalamene (13).
Figure VIII.5. Downfield region of COLOC spectrum of 2-hydroxy-8-o-angeloyloxycamene (13).
Figure VIII.6. 2D $^1$H NMR COSY spectrum of 2-Hydroxy-8α-hydroxycalamenene (42).
Figure VIII.7. 2D $^1$H NMR NOESY spectrum of 2-Hydroxy-8α-hydroxycalamenene (42).
Figure VIII.8. DEPT 90, 135 and BB 13C NMR of 2-Hydroxy-8o-hydroxyxalalenene (42).
Figure VIII.9.1. The 2D $^{13}$C-$^1$H HETCOR spectrum of 2-Hydroxy-8α-hydroxycalamene (42).
Figure VIII.9.2. Upfield region of HETCOR spectrum of 2-Hydroxy-8α-hydroxy calamene (42).
Fig. VIII.9.3. Effect of 2-Hydroxy-8α-angeloyloxycalamenene (13) on germination of conditioned *S. hermonthica* seeds occurring in Bida (A) and Kano (B) locations in Nigeria. Data are arc sine transformed relative percentage germination expressed as means ± S. E. of twelve replicates (P = 0.05). Control is GR 24 (8) dissolved in methanol (fig. con'd).
Fig. VIII.9.3. Effect of 2-Hydroxy-8α-angeloyloxy calamene (13) on germination of conditioned S. hermonthica seeds collected in Abuja location in Nigeria during the 1992 (C) and 1993 (D) harvesting seasons. Data are arcsine transformed relative percentage germination expressed as means ± S. E. of twelve replicates (P = 0.05). Control is GR 24 (8) dissolved in methanol.
Fig. VIII.9.4. Effect of 2-Hydroxy-8α-angeloyloxy-calamenene (13) on germination of conditioned S. aspera seeds occurring in Kano (E) location in Nigeria. Data are arc sine transformed relative percentage germination expressed as means ± S. E. of twelve replicates (P = 0.05). Control is GR 24 (8) dissolved in methanol.
CHAPTER IX

COMPOUNDS ISOLATED FROM THE WEST AFRICAN HARRISONIA ABYSSINICA OLIV. (SIMARUBACEAE)*

*This chapter contains some materials which have been reprinted with permission from Spectroscopy Letters.
IX. INTRODUCTION

IX.1. Limonoids. Limonoids, also referred to as tetranortriterpenoids, constitute a group of highly oxidized natural products known to occur in the Meliaceae, Rutaceae and Cneoraceae. These compounds apparently are formed by enzymatic oxidation and degradation of the tetracyclic triterpenoid precursor, euphol (43) (Akisanya et al., 1960). The limonoids have been classified on the basis of which of the four rings in the triterpene nucleus have been oxidized. The biosynthetic evolution of the limonoids has recently been proposed (Champagne et al., 1992). The citrus bitter principle limonin (44) was first isolated in 1960 (Arigoni et al., 1960), but intensive investigation of limonoids did not begin until the last decade (Hasegawa et al., 1984).

The separation of mixtures of limonoids by column chromatography is very difficult. These compounds are often very similar, unstable and may interconvert during isolation. High pressure liquid chromatography has been much used, but is not free from problems of isomerization. Separation is further complicated by the occurrence of mixtures of esters. Because of this, some compounds which have been isolated may be artifacts. When isolated, the limonoids may be of considerable molecular complexity, and the structure determination usually present formidable problems. Even in simple cases, the structure determination may present unexpected pitfalls for the unwary, and several methods of determining stereochemistry have failed when applied to limonoids (Arigoni et al., 1960). Moreover, the molecular conformation is not always readily predictable, and NMR spectroscopy of limonoids use of the Karplus equation has led to mistaken conclusions (Halsall et al., 1975).

From $^1$H NMR point of view, the rigid steroid framework of limonoids contain a relatively large number of functional groups that can exhibit diamagnetic anisotropy and would induce fields which can change the chemical shifts of neighboring protons in accord with their individual orientation to the functional group. These secondary fields can then be evaluated in a series of compounds where regular changes in structure have
been made. In many cases where overlapping bands occur, they can be separated by or shifted by taking the spectra in different solvents. Band shapes and coupling constants could thus be established with certainty. Some higher melting limonin derivatives are not sufficiently soluble in chloroform for good NMR structure. They would usually dissolve in mixtures of solvents, for example deuteriochloroform-dimethyl sulfoxide, deuteriochloroform-acetonitrile, and trifluoro acetic acid. More reliable has been the effect of substituents on the chemical shift of the nearby methyl groups (Jibodu et al., 1970). It has been shown that the presence of a hydroxy substituent causes a considerable downfield shift of the methyl substituents in a 1,3 diaxial relationship, while an acetooxy or other carbonyl containing substituent may or may not produce a similar shift depending on orientation effects. This can produce good evidence of stereochemistry, and in suitable cases, of the position of a substituent (Halsall et al., 1975). The general features of the NMR spectra of limonin (44) and its derivatives starting from the high field end of the spectrum can be summarized as follows:

i). Bands due to the C-methyl resonances.

ii). The absorption bands for the four protons at C-2 and C-6, α- to the carbonyl groups. For most limonin derivatives these bands are not interpretable.

iii). A sharp singlet due to the proton at C-15, adjacent to the epoxy group. This band sometimes overlap the band due to the proton at C-1.

ix). A broad band due to the protons on C-1 which in some cases is resolved into a symmetrical triplet.

v). An AB system from the protons on C-19, which in several derivatives collapses into a broadened singlet.

vi). A very slightly broadened singlet caused by the proton at C-17, adjacent to the furan ring.

vii). The structural changes in the D-ring is greatly influences the multiplicity of the furan absorption bands.
In most derivatives, structurally similar to limonin (44), the C-methyl resonance furthest downfield is that due to the C-methyl at C-13. The downfield shift of this methyl group is presumably due to the anisotropic effect of the furan ring. Since the gem-dimethyls at C-4 are attached to a carbon atom which is in turn attached to an ether oxygen, the chemical shift of such a group would be further downfield than that observed for the C-methyl at C-8. Thus, the assignment of the highfield C-methyl peak falls to the C-methyl group at C-8 largely by elimination.

The West African shrub *Harrisonia abyssinica* Oliv. (Simarubaceae) ("Msabubini" or "Mpapura-doko" in Swahili) is widely used in various folk remedies, including treatment for tuberculosis, fever, bubonic plague, haemorrhoid, snake-bite, etc (Hassanali et al., 1987). Four tetranortriterpenes, obacunone (45), pedonin (46), harrisonin (47) and 12β-acetoxyharrisonin (48), have so far been isolated from this shrub. The first two limonoids showed mild antifeedant activities against the larvae of the East African monophagous crop pest *Spodoptera exempta* (Kubo et al., 1976) and the third against the Southern armyworm, *Spodoptera eridania* (Hassanali et al., 1987). Our recent interest in comparative activities of *Striga* species seed germination stimulants from native Nigerian plants has led to an examination of the methanolic root extracts of *H. abyssinica*. The complete carbon-13 assignments of obacunone (45) could not be unambiguously assigned by previous workers (Okorie, 1982; Kubo et al., 1976), and we
now report the high field $^1$H and $^{13}$C spectral assignments of methyl groups and quaternary carbons (C-4, C-10 and C-13).

**IX.2. MATERIALS AND METHODS**

**IX.2.1. Structure Elucidation.** Mp measured on a Thomas Hoover apparatus (uncorr.). Mass spectrum were recorded on a Hewlett-Packard 5971 A GC/MS spectrometer. The IR spectrum was recorded on a Perkin-Elmer 1760x FT-IR spectrometer as a film on KBr plate using CHCl$_3$ solution.

One- or two-dimensional $^1$H- and $^{13}$C-NMR spectra were recorded on a Bruker AM 400 (400 MHz) spectrometer at room temperature. The spectrometer was locked on the deuterium signal of CDC$_3$. All experiments were performed on obacunone (45) samples of the same weight as ca. 10% CDC$_3$ solutions in 5-mm NMR tube (0.6 mL of solution used). All chemical shifts are expressed in $\delta$ (ppm) using TMS as internal standard ($\delta_{\text{TMS}} = 0$ ppm). Chemical shifts are reported in ppm on the $\delta$ scale. The following are the 2D NMR techniques employed: $^1$H-$^1$H correlation spectroscopy (COSY) (Aue *et al.*, 1976), NOESY (Odenhausen *et al.*, 1984), HETCOR ($J_{\text{CH}} = 125$) (Bax and Morris, 1981) and the long-range COLOC ($J_{\text{CH}} = 7$) (Kessler *et al.*, 1984).

The DEPT experiments (Doddrell *et al.*, 1982) were carried out with $\theta = 90^\circ$ and $135^\circ$. The quaternary carbons were determined by subtraction of these spectra from the broad band $^{13}$C NMR spectra. The standard pulse programs for the 2D NMR experiments employed were those provided by Bruker instruments, COSY.AU for $^1$H shift correlation, XHCORR.AU for CH-correlation with homonuclear $^1$H decoupling, COLOC.AU for long-range (3-bond) CH-correlation with polarization (D2 0.06 sec) set for 8 Hz and NOESY.AU for nOe difference. The $^1$H-$^1$H-COSY correlation and NOESY maps consisted of 256 X 1 K data points per spectrum, each composed of 32 transients. The HETCOR and COLOC sequences used were those described by Bax and Morris (1981) and Kessler *et al.* (1984), respectively. In both experiments, a 2-sec delay was allowed between each scan, and the coupling constant was optimized for $J=125$ Hz.
and 7 Hz, respectively. The final size of the data matrix was 2K X 2K. The number of acquisitions at each t₁ was 32 with 4 dummy acquisitions also being acquired. The HETCOR and COLOC correlation maps consisted of 256 X 4 K data points per spectrum, each composed of 64 transients. In all 1D and 2D experiments, the sample was spun.

IX.2.2. Isolation of Obacunone (45). Roots of H. abyssinica were collected near Moniya market, Ibadan, Nigeria on August 1, 1994. Authentication was done by Mr. C. W. Agyakwa in the Weed Science Section, International Institute of Tropical Agriculture (IITA). A voucher specimen is deposited in the IITA Herbarium (Voucher No. Rugutt 61). The air-dried powdered roots (50 g) were extracted with methanol (6 X 250 mL) at room temperature for 3 days. Each crude extract was filtered in a Buchner funnel. The extracts were combined and methanol evaporated under reduced pressure at low temperature (40°C). The resulting crude extract was shipped to the Chemistry laboratory at Louisiana State University (LSU) and stored in the refrigerator at 4°C until analysis. At LSU, the crude extract (1.1 g) was chromatographed on a VLC (Pelletier et al., 1986) column packed with 100 g silica gel (MN Kieselgel G) using ethyl acetate-hexane as eluent. The percentage of EtOAc in hexane was gradually increased (0:50, 5:45, 10:40, 15:35, 25:25, 35:15, 45:5, 50:0). Forty fractions of 10 mL each were collected and monitored by TLC (Fischer, 1991) performed on precoated MN Sil-G 25 UV254 plates having a layer thickness of 0.25 mm. The TLC plates were developed with 35% ethyl acetate in hexane and made visible by spraying with a solution prepared by dissolving CoCl₂.6H₂O (2 g) in 10% aqueous H₂SO₄ (100 mL) and heating until a colored spot appeared. The homogeneous fractions 30-39 eluted with 35% EtAOc-hexane were combined and evaporated to afford 5.1 mg of obacunone (45) as a yellow fluffy solid [Rf 0.25 (SiO₂, EtOAc:Hexane, 2:6); MS 454 [M⁺] and mp 227-231° (lit. (Kubo et al., 1976) 226-228°)].
IX.2.3. Isolation of Pedonin (46), Harrisonin (47) and 12β-acetoxyharrisonin (48). Air-dried roots (30 g) of the same plant was extracted consecutively with solvents of increasing polarity (hexane, ether, dichloromethane, methanol and water). The dichloromethane extract was concentrated under reduced pressure to give a residue (1.2 g) which was chromatographed on 350 g silica gel with benzene and increasing amounts of the ether in benzene (0:50, 5:45, 10:40, 15:35, 45:5 and 50:0, 50 mL each). The fraction eluted with benzene-ether (45:5), (5:45) and (10:40) afforded (30 mg) pedonin (46) \{C_{27}H_{32}O_9, \text{Mr.} 500.5, \text{mp.} 258-260°C; R_f 0.53, \text{hexane-ether,} 2:1\}, (15 mg) harrisonin (47) \{C_{27}H_{32}O_{10}, \text{Mr.} 517, \text{mp.} 154-155°C; R_f 0.40, \text{hexane-ether,} 2:1\} and (8 mg) 12β-acetoxyharrisonin (48) \{C_{29}H_{34}O_{12}, \text{Mr.} 575, \text{mp} 252-254°C; R_f 0.42, \text{hexane-ether,} 2:1\}, respectively. All these limonoids were isolated as white powder.

IX.2.4. Isolation of (+)-ononitol (49). The methanolic extract (0.5 g) of the roots was chromatographed on a silica gel column. The fraction obtained by elution with 1:1 acetone-methanol afforded 90 mg of colorless prism-like crystals which were identified by X-ray structure analysis to be (+)-ononitol (49) \{4-O-methyl-myoinositol-hydrate, C_{7}H_{14}O_{6}.H_2O, \text{mp} 186-188°C; R_f 0.46, \text{hexane-methanol,} 1:2\}.

The crystal structure determination was carried out by Frank R. Fronczek. A colorless crystal fragment of dimensions 0.30 x 0.40 x 0.52 mm was used for data collection on an Enraf-Nonius CAD4 diffractometer equipped with CuKα radiation (λ = 1.54184 Ǻ), and a graphite monochromator. Crystal data are: C_{7}H_{14}O_{6}.H_2O \text{Mr.} 212.2, \text{triclinic space group P1, a = 5.079(1), b = 6.604(1), c = 7.758(1)Å, α = 106.00(1), β = 93.49(1), γ = 109.10(1)°, V = 233.0(1)Å³, Z = 1, d_c = 1.512 g cm}^{-3}, T = 23°C. Intensity data were measured by ω-2θ scans of variable rate. A hemisphere of data was collected within the limits 2 < θ < 75°. Data reduction included corrections for background, Lorentz, polarization, and absorption effects. Absorption corrections (μ = 11.4 cm}^{-1}) were based on ψ scans, with minimum relative transmission coefficient.
Coordinates of Kailiang et al. (1988) were used as a starting model and refined using the Enraf-Nonius MolEN programs. Refinement was by full-matrix least squares, treating nonhydrogen atoms anisotropically and hydrogen atoms isotropically. Convergence was achieved with $R = 0.054$ for 189 variables. Maximum residual electron density was 0.63 eÅ$^{-3}$.

**IX.2.5. Bioassays.** Test solutions were prepared by dissolving 10 mg of obacunone (45), pedonin (46), harrisonin (47) and (+)-ononitol (49) in 0.1 mL dimethyl-sulfoxide (DMSO) as the initial solvent carrier followed by diluting with 9.9 mL sterile distilled water to a final concentration of 1,000 mgL$^{-1}$. Other test solutions (i.e. 100, 10 mgL$^{-1}$) were prepared by diluting with sterile distilled water. Each test solution (600 μL) was added separately to a sterile glass petri dish lined with Whatman No. 1 filter paper. Stimulatory activity of test solutions was assayed using conditioned *S. hermonthica* seeds collected in 1993 in Bida, Nigeria. The seeds were surface disinfested by immersion in 1% NaOCl for a brief period and then rinsed several times with sterile distilled water. Treatments consisted of three replicates of about 360 seeds in twelve evenly spaced 5 mm diameter disks (per petri dish). Six replicates of each treatment were arranged and the experiment repeated three times. Treated seeds were kept in the dark in an incubator at 28°C. Germination (radicle protrusion through the seed coat) of all assay seeds were recorded after 24 h. Germination data were transformed to arc sine and compared to controls using single-degree-of-freedom contrasts with the general linear models procedure of the Statistical Analysis System (SAS, 1988) programs.

**IX.3. RESULTS AND DISCUSSIONS.** Obacunone (45, C$_{26}$H$_{30}$O$_7$), an optically active limonoid, has previously been isolated from the West (Okorie, 1982) and the East (Kubo et al., 1976) African *H. abyssinica* plants and also from citrus species (Emerson, 1948) as one of their bitter principles. This compound contains two lactone rings, which can be opened reversibly with alkali, $\alpha$ $\beta$-substituted furan residue, a
ketonic oxygens present in the 6-membered ring and one epoxide (Barton and Pradhan, 1961). The assignment of all proton and most carbon of NMR signals of obacunone (45) have been made partly through comparison of the chemical shifts with the published data of the same compound (Okorie, 1982; Kubo et al., 1976) and partly through high field 1D and 2D experiments (Table IX.1).

Obacunone (45) exhibited IR bands which were typical of a limonoid skeleton (α β-substituted furan at $\nu_{\text{max}} \text{cm}^{-1}$: 1501 and 878, three CO bands at 1734, 1711 and 1645, the last band being due to an α,β-unsaturated C=O). The $^{13}$C-NMR spectra reported in Table IX.1 confirm the presence of 26 carbon atoms, eleven of which show resonances in the chemical shift range of $\delta > 77$ ppm. The DEPT spectrum (Figure IX.1) exhibited 5 methyls, 3 methylenes and 9 methine signals, while the remaining 9 signals in the broad-band spectrum were due to quaternary carbon atoms. The downfield signals are three singlets for the quaternary carbonyl carbons C-7, C-16 and C-3. The upfield absorptions of $\delta < 77$ are characterized by four singlets (C-14, C-8, C-10 and C-13), three doublets (C-5, C-9 and C-15), three triplets (C-6, C-11 and C-12), and five quartets for aliphatic C-methyls (C-18, C-19, C-28, C-29 and C-30). The methyl and quaternary carbon $^{13}$C-NMR signals were unambiguously assigned by employing the conventional 1D and 2D NMR techniques.

The high field $^1$H-NMR of obacunone (45) showed two sharp singlets, one at 3.65 ppm due to the proton at the epoxy carbon C-15, and the other at 5.45 ppm, a slightly broadened singlet caused by the proton at C-17, adjacent to the furan ring. The C-15 proton is clearly influenced by the anisotropic effect of the C-7 keto carbonyl and must be deshielded by the keto group in order to account for the observed downfield shift, when compared to shift values of analogues without the C-7 carbonyl group (Dreyer, 1965). Two doublets at 6.51 and 5.96 ppm due to the protons on a disubstituted double bond (C-1 and C-2) showed an AB-type system. The contour plot of the $^1$H-$^1$H-COSY-45 spectrum (Figure IX.2) showed that H-1 resonating at 6.51 ppm
was scalar-coupled only with H-2 proton resonating at 5.96 ppm and no further couplings to other protons was indicated. Cross peaks between H_3-18 and H-15α occurred in the COSY spectrum. However, the COSY map contained ambiguities of the remaining methyl resonances which could only be eliminated by identification of the spatial proximities as detected in 2D NOESY-45 spectrum (Figure IX.3). The ^1H NMR spectrum of 45 showed three methyl proton signals at 1.12, 1.24, 1.45 ppm, and two overlapping absorptions at 1.46 ppm, which showed correlation peaks in the HETCOR map (Figure IX.4) with five carbon quartets in the ^13C-NMR spectrum at 16.4, 22.1, 20.5, 26.8 and 32.5 ppm. The methyl resonance at 1.12 ppm was assigned to H_3-18 due to fact that in the COSY spectrum showed cross peaks with H-17α; in the NOESY spectrum it showed spatial interactions with three protons, H-9α, H-21 and H-22 and in the COLOC map (Figure IX.5), a correlation peak with the quaternary carbon C-14 was observed. The methyl singlet at 1.24 ppm was ascribed to H_3-30 because it showed a cross peak with C-14 carbon in COLOC spectrum and spatial interactions with H-15α, H-6β, and one or both of the methyl groups at 1.46 ppm in the NOESY spectrum. The methyl proton resonance at 1.45 ppm showing spatial interactions with H-5α and H-6α and was assigned to H_3-28. The quaternary carbon resonance at 52.9 ppm was assigned C-8 because it showed three-bond coupling to H_3-30 methyl in the COLOC spectrum. The quaternary carbon resonance at 83.4 ppm showed correlation peaks with H_3-28 and H_3-29 methyl groups in the COLOC spectrum and was assigned to C-4.

The comparison between COSY and NOESY spectra (Figures IX.2 and IX.3) revealed that several J coupling cross peaks have been effectively suppressed in the NOESY spectrum (e.g. the H-17β, -H_3-18; H-6β, -H-5α; H-6β, -H-6α; and H-9α, -H-11α,β, at \{f_1;f_2\} = {5.45, 1.12}, {2.90, 2.29}, {2.90, 2.60}, {2.15, 1.8 - 1.95} (Figure IX.2), respectively. The mixing time of 800 msec allowed for the spatial detection of several cross peaks, the most revealing of which connect H_3-30 with H-15α, H-6β and H_3-19, H-6β with H_3-19 and H_3-29, H-6α with H_3-28, and H_3-18 with H-
21, H-9α, H-15α and H-17β. The integration intensity of the peaks overlapping in the region 1.8-1.95 ppm indicated four protons, which, on the basis of COSY and NOESY spectra were assigned to H-11 and H-12 protons. A model was created using PCMODEL (MMX- force field energy minimization) and all experimental nOEs were in agreement with the model.

The high field ¹H NMR measurements of obacunone (45) indicated marginal differences in coupling constants with those reported (Okorie, 1982; Kubo et al., 1976). The arrangements of a relatively large number of functional groups in a framework of rigid stereochemistry causes the protons of 45 to be shielded to different extents. Thus many of the protons appeared in distinctive regions of the spectrum and there were few overlapping bands. Our implementation of high field 2D NMR techniques reduced misassignments in both ¹H and ¹³C spectra, which are mainly due to overlap of the signals in the upfield regions.

Complete and unambiguous assignments of all the protons and carbon signals of pedonin (46), harrisonin (47) and 12β-acetoxyharrisonin (48) and (+)-ononitol (49) were obtained through COSY, NOESY, HETCOR and COLOC experiments (Figures IX.6-20). Our NMR data were in close agreement with those reported by Kubo et al. (1976) and Hassanali et al. (1987). However, NOESY and COLOC spectra of obacunone (45) and pedonin (46) strongly indicated that the previous assignments of ¹³C and ¹H chemical shifts needs revision.

Pedonin (46) belongs to a growing group of limonoids in which one or more of the rings of the cyclopentenophenanthrene skeleton are cleaved (Connolly, 1983). The tetranortriterpenoid nature of pedonin (46) and its close relationship to obacunone (45) and harrisonin (47) was apparent from NMR spectroscopic data. The ¹H NMR spectrum of 46 (Figure IX.6) showed resonances for five tertiary methyl groups (0.98, 1.24, 1.38, 1.58 and 1.64 ppm), a dissubstituted double bond (6.35 and 6.17, d, J = 10 Hz, H-1 and H-2), the characteristics of α β-substituted furan (6.94, 7.34 and 8.59 ppm) and a
secondary epoxidic proton (3.75, s, H-15). A major difference between the $^1\text{H}$ NMR spectrum of 46 and those of obacunone (45) and harrisonin (47) (Figure IX.13) was the downfield shift (8.59 ppm) of one of the furan protons, suggesting the presence of a deshielding anisotropic effect of a C-17 carbonyl group. The $^{13}\text{C}$ NMR spectrum indicated that two of the four carbonyl groups were ketonic and the other two were either lactonic or ester, and confirmed the presence of an $\alpha,\beta$-unsaturated furan and a secondary, tertiary ether unit.

The $^1\text{H}$ NMR spectrum of harrisonin (47) (Figure IX.13) differs from that of obacunone (45) in that the former lacks the H-5/H-6α/H-6β three proton system in the region 3.00-2.00 ppm, and that 4.30 ppm peak (the H-15α) in 47 is downfield in comparison to the 3.65 ppm peak in 45 (Table IX.1). The tetranortriterpenoid nature of 12β-acetoxyharrisonin (48), which has an $\alpha,\beta$-epoxy-δ-lactone group in the ring D and an $\alpha,\beta$-unsaturated lactone group in ring A was clarified by $^1\text{H}$- and $^{13}\text{C}$-NMR analyses. The only difference between harrisonin (47) and 12β-acetoxyharrisonin (48), was the presence of an additional acetoxy group. The appearance of a CH-OAc signal at 4.76 ppm ($^1\text{H}$ NMR, Figure IX.16) and a methine peak at 72.9 ppm ($^{13}\text{C}$ NMR, Figure IX.19) limited the attachment of the acetoxy group to either C-11 or C-12. The assignment of the acetoxy group at C-12 was based on the chemical shifts of the adjacent carbons. Namely, C-11 and the quaternary carbon (C-13) peaks at 15.3 and 39.7 ppm, respectively, in harrisonin (47) were shifted downfield to 26.1 and 42.7 ppm in 48 due to the well-known acetylation or esterification on the $\beta$-carbon. Unambiguous assignment of 12β-acetoxy group configuration was based on the analyses of $^1\text{H}$ NMR data as well as COSY (Figure IX.17) and NOESY (Figure IX.18) spectra. The 14 Hz and the 5 Hz J values of the H-9α 2.96 ppm signal showed that the 2.35 ppm signal (J$_{9,11}$ = 5 Hz) and 1.76 ppm signal (J$_{9,11}$ = 14 Hz) should be assigned to H-11α (eq) protons, respectively; since the 4.75 ppm H-12 peak is coupled to H-11β and H-11α by 1 Hz and the 7 Hz, respectively, its configuration is $\alpha$, i.e., the 12-acetoxy group is $\beta$-
oriented. The two hydroxyl groups of the 12β-acetoxylharrisonin (48) are both involved in hydrogen bonding as evidenced by two sharp 1H NMR singlets at 3.65 and 5.11 ppm (in CDCl₃).

The stereoscopic illustration of (+)-ononitol (49) is shown below. The atomic parameters are listed in Table IX.2. Bond lengths, selected bond lengths and torsion angles are listed in Tables IX.3.1-3, respectively. It is worth noting that there is no intramolecular hydrogen bond in (+)-ononitol (49), but intermolecular hydrogen bonds exist between water molecule and hydroxyl group as well as between hydroxyl groups (Kailiang et al., 1988). The six membered ring of 49 takes a chair conformation and the conformation of the 4-O-methyl-myoinositol molecule is described by the torsion angles given in Table IX.3.3. All C-O bonds are equitorial-bonds, except the bond C(6)-O(6) which is axial-bond. The C-C and C-O distances falling in the range of 1.520-1.530 and 1.420-1.430 Å respectively, are typical of single bonds. The absolute configuration of (+)-ononitol (49) is yet to be determined. Preliminary bioassay has shown that 49 has some curative effect on blood vessel dilatation of the heart (Kailiang et al., 1988).

IX.3.1. Effect of (+)-ononitol (49) and Limonoids on Germination of S. hermonthica Seeds. Preliminary bioassays indicated that obacunone (45), pedonin (46), harrisonin (47) and (+)-ononitol (49) induce germination of conditioned Striga hermonthica seeds collected in Bida during the 1993 harvesting season (Table IX.4). This is encouraging because many limonoids are potentially available in very large quantities. For example, the timber of some species may yield 1% of an isolated crystalline limonoid; while a single tree of Entadrophragma angolense may contain more than 100 Kg. of gedunin (50), much of it easily recoverable from timber mill offcuts (Lavie and Troke, 1967).
Table IX.1. $^{13}$C-$^1$H correlation of obacunone (45) (400 MHz, CDCl$_3$)

<table>
<thead>
<tr>
<th>$\delta^{13}$C</th>
<th>Mult.$^a$</th>
<th>$\delta$attached$^b$H</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>156.7</td>
<td>CH</td>
<td>6.51</td>
<td>1</td>
</tr>
<tr>
<td>123.0</td>
<td>CH</td>
<td>5.96</td>
<td>2</td>
</tr>
<tr>
<td>166.6</td>
<td>$C^b$</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>83.4</td>
<td>$C^b$</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>53.3</td>
<td>CH</td>
<td>2.29</td>
<td>5</td>
</tr>
<tr>
<td>39.9</td>
<td>CH$_2$</td>
<td>$H_\alpha = 2.60; H_\beta = 2.96$</td>
<td>6</td>
</tr>
<tr>
<td>207.4</td>
<td>$C^b$</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>52.9</td>
<td>$C^b$</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>49.2</td>
<td>CH</td>
<td>2.15</td>
<td>9</td>
</tr>
<tr>
<td>43.1</td>
<td>$C^b$</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>19.4$^c$</td>
<td>CH$_2$</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>26.8$^c$</td>
<td>CH$_2$</td>
<td>${ 1.8-1.95$</td>
<td>12</td>
</tr>
<tr>
<td>37.3</td>
<td>$C^b$</td>
<td>-</td>
<td>13</td>
</tr>
<tr>
<td>68.5</td>
<td>$C^b$</td>
<td>-</td>
<td>14</td>
</tr>
<tr>
<td>57.3</td>
<td>CH</td>
<td>3.65</td>
<td>15</td>
</tr>
<tr>
<td>166.9</td>
<td>$C^b$</td>
<td>-</td>
<td>16</td>
</tr>
<tr>
<td>84.0</td>
<td>CH</td>
<td>5.45</td>
<td>17</td>
</tr>
<tr>
<td>22.1</td>
<td>CH$_3$</td>
<td>1.12</td>
<td>18</td>
</tr>
<tr>
<td>26.8</td>
<td>CH$_3$</td>
<td>1.46</td>
<td>19</td>
</tr>
<tr>
<td>120.1</td>
<td>$C^b$</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>141.0</td>
<td>CH</td>
<td>7.42</td>
<td>21</td>
</tr>
<tr>
<td>109.7</td>
<td>CH</td>
<td>6.36</td>
<td>22</td>
</tr>
<tr>
<td>143.2</td>
<td>CH</td>
<td>7.40</td>
<td>23</td>
</tr>
<tr>
<td>20.5</td>
<td>CH$_3$</td>
<td>1.45</td>
<td>28</td>
</tr>
<tr>
<td>16.4</td>
<td>CH$_3$</td>
<td>1.46</td>
<td>29</td>
</tr>
<tr>
<td>32.9</td>
<td>CH$_3$</td>
<td>1.24</td>
<td>30</td>
</tr>
</tbody>
</table>

$^a$ Carbon multiplicities determined through DEPT experiments. $^b$ Assignments based on COLOC experiment. $^c$ Values within the same column may be interchanged.
Obacunone (45)

Pedonin (46)

H Harrisonin (47)

OAc 12β-acetoxyharrisonin (48)

Gedunin (50)
Table IX.2. Coordinates and equivalent isotropic thermal parameters

<table>
<thead>
<tr>
<th>atom</th>
<th>( x )</th>
<th>( y )</th>
<th>( z )</th>
<th>( B_{eq}(\text{Å}^2) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2.43(2)</td>
</tr>
<tr>
<td>O2</td>
<td>1.1317(3)</td>
<td>0.9603(2)</td>
<td>0.6454(2)</td>
<td>2.38(2)</td>
</tr>
<tr>
<td>O3</td>
<td>0.8820(3)</td>
<td>0.5325(2)</td>
<td>0.3772(2)</td>
<td>2.45(2)</td>
</tr>
<tr>
<td>O4</td>
<td>0.819(3)</td>
<td>0.1515(2)</td>
<td>0.5010(2)</td>
<td>2.96(3)</td>
</tr>
<tr>
<td>O5</td>
<td>0.6622(3)</td>
<td>0.1862(2)</td>
<td>0.8556(2)</td>
<td>2.48(2)</td>
</tr>
<tr>
<td>O6</td>
<td>0.5403(3)</td>
<td>0.5892(2)</td>
<td>0.9094(2)</td>
<td>2.27(2)</td>
</tr>
<tr>
<td>C1</td>
<td>1.0127(3)</td>
<td>0.7965(2)</td>
<td>0.8840(2)</td>
<td>1.74(3)</td>
</tr>
<tr>
<td>C2</td>
<td>0.9390(3)</td>
<td>0.7727(2)</td>
<td>0.6838(2)</td>
<td>1.70(2)</td>
</tr>
<tr>
<td>C3</td>
<td>0.9584(3)</td>
<td>0.5538(2)</td>
<td>0.5633(2)</td>
<td>1.74(3)</td>
</tr>
<tr>
<td>C4</td>
<td>0.7658(4)</td>
<td>0.3473(3)</td>
<td>0.6069(2)</td>
<td>1.88(3)</td>
</tr>
<tr>
<td>C5</td>
<td>0.8364(3)</td>
<td>0.3728(2)</td>
<td>0.8082(2)</td>
<td>1.78(3)</td>
</tr>
<tr>
<td>C6</td>
<td>0.8231(3)</td>
<td>0.5934(3)</td>
<td>0.9298(2)</td>
<td>1.77(3)</td>
</tr>
<tr>
<td>C7</td>
<td>0.6024(6)</td>
<td>0.0091(4)</td>
<td>0.3449(3)</td>
<td>3.65(5)</td>
</tr>
<tr>
<td>O1W</td>
<td>1.3213(3)</td>
<td>0.4309(2)</td>
<td>1.1991(2)</td>
<td>2.68(3)</td>
</tr>
<tr>
<td>H1</td>
<td>0.859(7)</td>
<td>1.024(5)</td>
<td>0.964(4)</td>
<td>3.2(6)</td>
</tr>
<tr>
<td>H2</td>
<td>1.039(8)</td>
<td>1.017(6)</td>
<td>0.597(5)</td>
<td>4.0(7)</td>
</tr>
<tr>
<td>H3</td>
<td>1.023(8)</td>
<td>0.504(6)</td>
<td>0.309(5)</td>
<td>4.3(8)</td>
</tr>
<tr>
<td>H5</td>
<td>0.500(9)</td>
<td>0.126(6)</td>
<td>0.786(6)</td>
<td>6.1(1)</td>
</tr>
<tr>
<td>H6</td>
<td>0.465(4)</td>
<td>0.548(3)</td>
<td>0.978(3)</td>
<td>1.0(4)</td>
</tr>
<tr>
<td>H1a</td>
<td>1.193(5)</td>
<td>0.793(3)</td>
<td>0.907(3)</td>
<td>1.1(3)</td>
</tr>
<tr>
<td>H2a</td>
<td>0.772(7)</td>
<td>0.782(5)</td>
<td>0.662(4)</td>
<td>3.1(6)</td>
</tr>
<tr>
<td>H3a</td>
<td>1.137(4)</td>
<td>0.558(3)</td>
<td>0.582(3)</td>
<td>0.5(3)</td>
</tr>
<tr>
<td>H4a</td>
<td>0.560(8)</td>
<td>0.342(6)</td>
<td>0.561(5)</td>
<td>4.4(8)</td>
</tr>
<tr>
<td>H5a</td>
<td>1.017(5)</td>
<td>0.372(4)</td>
<td>0.831(3)</td>
<td>1.9(4)</td>
</tr>
<tr>
<td>H6a</td>
<td>0.872(5)</td>
<td>0.606(4)</td>
<td>1.038(3)</td>
<td>1.6(4)</td>
</tr>
<tr>
<td>H7a</td>
<td>0.44(1)</td>
<td>-0.010(7)</td>
<td>0.377(7)</td>
<td>7(1)</td>
</tr>
<tr>
<td>H7b</td>
<td>0.63(1)</td>
<td>-0.134(8)</td>
<td>0.293(7)</td>
<td>6(1)</td>
</tr>
<tr>
<td>H7c</td>
<td>0.63(2)</td>
<td>0.10(1)</td>
<td>0.25(1)</td>
<td>13(2)</td>
</tr>
<tr>
<td>H1W</td>
<td>1.254(7)</td>
<td>0.293(5)</td>
<td>1.129(5)</td>
<td>3.4(6)</td>
</tr>
<tr>
<td>H2W</td>
<td>1.45(1)</td>
<td>0.442(7)</td>
<td>1.251(7)</td>
<td>5.3(9)</td>
</tr>
</tbody>
</table>

\[ B_{eq} = 8\pi^2/3 \sum_i \sum_j U_{ij} a_i^* a_j^* a_i \cdot a_j \]
<table>
<thead>
<tr>
<th>Table IX.3.1. Selected bond lengths (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1-C1</td>
</tr>
<tr>
<td>O2-C2</td>
</tr>
<tr>
<td>O3-C3</td>
</tr>
<tr>
<td>O4-C4</td>
</tr>
<tr>
<td>O4-C7</td>
</tr>
<tr>
<td>O5-C5</td>
</tr>
<tr>
<td>O6-C6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table IX.3.2. Selected bond angles (deg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4-O4-C7</td>
</tr>
<tr>
<td>O1-C1-C2</td>
</tr>
<tr>
<td>O1-C1-C6</td>
</tr>
<tr>
<td>C2-C1-C6</td>
</tr>
<tr>
<td>O2-C2-C1</td>
</tr>
<tr>
<td>O2-C2-C3</td>
</tr>
<tr>
<td>C1-C2-C3</td>
</tr>
<tr>
<td>O3-C3-C2</td>
</tr>
<tr>
<td>O3-C3-C4</td>
</tr>
<tr>
<td>C2-C3-C4</td>
</tr>
</tbody>
</table>
Table IX.3.3. Selected torsion angles (deg.)

<table>
<thead>
<tr>
<th>Torsion Angle</th>
<th>Torsion Angle</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C7-O4-C4-C3</td>
<td>O1-C1-C2-O2</td>
<td>-102.7(2)</td>
</tr>
<tr>
<td>C7-O4-C4-C5</td>
<td>O1-C1-C2-C3</td>
<td>137.3(2)</td>
</tr>
<tr>
<td>C6-C1-C2-O2</td>
<td>O1-C1-C6-O6</td>
<td>-176.8(1)</td>
</tr>
<tr>
<td>C6-C1-C2-C3</td>
<td>O1-C1-C6-C5</td>
<td>-56.4(2)</td>
</tr>
<tr>
<td>C2-C1-C6-O6</td>
<td>O2-C2-C3-O3</td>
<td>-65.4(2)</td>
</tr>
<tr>
<td>C2-C1-C6-C5</td>
<td>O2-C2-C3-C4</td>
<td>56.1(2)</td>
</tr>
<tr>
<td>C2-C3-C4-O4</td>
<td>O3-C3-C4-O4</td>
<td>-175.1(2)</td>
</tr>
<tr>
<td>C2-C3-C4-C5</td>
<td>O3-C3-C4-C5</td>
<td>-56.4(2)</td>
</tr>
<tr>
<td>C1-C2-C3-O3</td>
<td>O4-C4-C5-O5</td>
<td>178.6(1)</td>
</tr>
<tr>
<td>C1-C2-C3-C4</td>
<td>O4-C4-C5-C6</td>
<td>57.0(2)</td>
</tr>
<tr>
<td>C3-C4-C5-O5</td>
<td>O5-C5-C6-O6</td>
<td>179.6(2)</td>
</tr>
<tr>
<td>C3-C4-C5-C6</td>
<td>O5-C5-C6-C1</td>
<td>54.7(2)</td>
</tr>
<tr>
<td>C4-C5-C6-O6</td>
<td>C4-C5-C6-C1</td>
<td>65.4(2)</td>
</tr>
</tbody>
</table>

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
Table IX.4. Percentage germination of conditioned *Striga hermonthica* seeds treated with aqueous solutions of obacunone (45), pedonin (46), harrisonin (47) and (+)-ononitol (49).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (Moles/L)</th>
<th>% Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obacunone (45)</td>
<td>2.3 x 10^{-3} M</td>
<td>85 (66.9)*</td>
</tr>
<tr>
<td></td>
<td>2.3 x 10^{-4} M</td>
<td>90 (72.5)</td>
</tr>
<tr>
<td></td>
<td>2.0 x 10^{-5} M</td>
<td>22 (27.9)</td>
</tr>
<tr>
<td>Pedonin (46)</td>
<td>2.0 x 10^{-3} M</td>
<td>70 (56.9)</td>
</tr>
<tr>
<td></td>
<td>2.0 x 10^{-4} M</td>
<td>80 (63.7)</td>
</tr>
<tr>
<td></td>
<td>2.0 x 10^{-5} M</td>
<td>18 (24.9)</td>
</tr>
<tr>
<td>Harrisonin (47)</td>
<td>1.9 x 10^{-3} M</td>
<td>28 (31.8)</td>
</tr>
<tr>
<td></td>
<td>1.9 x 10^{-4} M</td>
<td>30 (33.1)</td>
</tr>
<tr>
<td></td>
<td>1.9 x 10^{-5} M</td>
<td>12 (19.9)</td>
</tr>
<tr>
<td>Ononitol (49)</td>
<td>4.7 x 10^{-3} M</td>
<td>86 (68.2)</td>
</tr>
<tr>
<td></td>
<td>4.7 x 10^{-4} M</td>
<td>98 (82.1)</td>
</tr>
<tr>
<td></td>
<td>4.7 x 10^{-5} M</td>
<td>26 (29.9)</td>
</tr>
</tbody>
</table>

* Figures in parentheses are arc sine transformations.
Figure IX.1. DEPT 90, 135 and BB $^{13}$C NMR spectra of obacunone (45).
Figure IX.2. 2D $^1$H NMR COSY of obacunone (45).
Figure IX.3. 2D $^1$H NMR NOESY spectrum of obacunone (45).
Figure IX.5. COLOC spectrum of obacunone (45).
Figure IX.7. 2D $^1$H NMR COSY spectrum of pedonin (46).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
Figure IX.8. 2D $^1$H NMR NOESY spectrum of pedonin (46).
Figure IX.10. DEPT 90, 135 and BB $^{13}$C NMR spectra of pedonin (46).
Figure IX.12. 2D 13C-1H COLOC spectrum of pedomin (46).
Figure IX.13. 1H NMR spectrum of harrisonin (47).
Figure IX.14. Downfield region of COSY spectrum of harrisonin (47).
Figure IX.15. 2D $^1$H NMR NOESY spectrum of harrisonin (47).
Figure IX.16. $^1$H NMR spectrum of 12β-acetoxyharrisonin (48).
Figure IX.17. 2D $^1$H NMR COSY spectrum of 12β-acetoxyharrisonin (48).
Figure IX.18. 2D $^1$H NMR NOESY spectrum of 12$\beta$-acetoxyharrisonin (48).
Figure IX.19. BB 13C NMR spectrum of 12β-acetoxyharrison (48).
Figure IX.20. DEPT 90, 135 and BB 13C NMR spectrum of 12β-acetoxyharrisontin (48).
CHAPTER X

FUTURE RESEARCH
The five classical plant hormones, namely ethylene (1), auxins (51), cytokinins (52), gibberellins (53) and abscissic acid (54), are responsible for the control of plant development and the integration of the activities of the different plant organs. From our rather simple approach mainly based on exogenous treatment of conditioned *Striga* spp. seeds with germination stimulants, we have shown that germination occurs over a broad concentration range suggesting that the stimulants are a new class of hormones. However, the central question which remains largely unanswered is: how far does the regulation of germination arise from modulation by these "exogenous" hormones?. Accurate measurements (Weiler, 1984) of the concentrations of hormones in *Striga* spp. seeds will provide useful information. Because *Striga* spp. seeds contain some of the compounds also occurring in host plants, we cannot rule out the possibility of a specific gene-for-gene relationships which may well occur as the rule rather than the exception in host:parasite systems (Person, C., 1959).

The studies in the isolation and structure elucidation of germination stimulants from host and nonhost plants yielded an array of compounds. There appears to be reason for confidence that our screening of plants will yield sorely-needed templates for the synthesis of potentially superior germination stimulants. Data on active concentration of germination stimulants in the soil needs to be determined by studying the rate of input to the environment (i.e. leachate, root exudates, or decomposition and leaching of plant residues), absorption and adsorption by *Striga* seeds, fixation by soil components, and leaching and microbiol degradation (Huang *et al.*, 1977). Moreover, studies on the stability of germination stimulants enhanced by entrapping or encapsulating in a biodegradable polymeric matrix (Shasha *et al.*, 1981) or cyclodextrins (Wang and Warner, 1993) which slowly releases the germination stimulant during biodegradation is needed.
REFERENCES


204

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


Hassanali, A.; Bentley, M. D.; Slawin, M. Z.; Williams, D. J.; Shephard, R. N.; Chapya, A. W. Pedonin, a spiro tetranortriterpenoid insect antifeedant from Harrisonia abyssinica. Phytochemistry, 1987, 26, 573-575.


Heftmann, E. Functions of Sterols in Plants. Lipids, 1971, 6, 128-133.


Hoshikawa, K. Significance of legume crops in improving the productivity and stability of cropping systems. Phosphorus Nutrition of Grain Legumes in the Semi-Arid...


Jansen, E. F. Metabolism of labelled ethylene in avocado. II. Benzene and toluene from ethylene-^14^C; benzene from ethylene-^3^H. *J. Biol. Chem.*, 1964, 238, 1664-1667.


Sand, P. F.; Manley, J. D. The witchweed eradication program survey, regulatory and control. In: P. F. Sand, R. G. Westerbrooks, eds. *Witchweed Research and


Vail, S. L.; Dailey, O. D.; Blanchard, E. J.; Pepperman, A. B.; Riopel, J. L. Terpenoid precursors of strigol as seed germination stimulants of Broomrape (*Orobanche*...
ramosa) and witchweed (Striga asiatica). J. Plant Growth Regul., 1990, 9, 77-83.


March 8, 1996

Mr. Joseph K. Rugutt
Department of Chemistry
Louisiana State University
Box C-12, Choppin Hall
Baton Rouge, LA 70803

Dear Mr. Rugutt:

Permission is granted to use any material published in your article "Carbon-13 Assignments and Revision of the Stereostructures of the Cadinanes 2-Hydroxy-8α-angeloyolxycalamene and 2-Hydroxy-8α-hydroxycalamene" and $^{13}$C-NMR assignments for tertiary methyls and quaternary carbons of the limonoid obacunone" by J.K. Rugutt in your Ph.D. Thesis.

Very truly yours,

Dr. J.W. Robinson
Professor of Chemistry

JWR/jh
VITA

Joseph Kipronoh Rugutt was born in Kericho District, Kenya on December 29, 1964 to the Christian parents, Mr. Thomas Kiprugutt arap Kamoing and Mrs. Rodah Chepmabwai Kamoing. He grew up in Keongo Village and attended Keongo Primary School. After graduating in 1979, he was admitted to St. Patrick’s High School Iten in Elgeiyo-Marakwet District where he had a uniquely successful career. At Iten, Mr. Rugutt was involved in sports, especially volleyball and football. He was an excellent leader and was selected the Deputy School Captain and School Captain in 1982 and 1984, respectively. He won several awards for outstanding performances in academic and leadership. After graduating from St. Patrick’s Iten, Mr. Rugutt joined National Youth Service (NYS) Pre-university Training in Gilgil, Naivasha. At NYS, he was selected the Commander in charge of Twiga Barracks which earned him President Daniel Arap Moi’s Best Recruit Award. In 1986, Mr. Rugutt joined Moi University, Eldoret for a Bachelor of Science degree in Chemistry and was employed as a teaching assistant after graduating. At Moi University Mr. Rugutt met and married his beautiful wife, Janny Chepkemoi Koske who is currently studying Computer Science at Louisiana State University. On August 18, 1993 the beautiful Ms. Elizabeth Chepchumba was born to the Rugutt’s family. In January 1991 Mr. Rugutt enrolled at Louisiana State University where he taught CHEM 1212 and CHEM 2364. He was considered one of the best teaching assistants in the Department of Chemistry. In 1994, Mr. Rugutt was the recipient of the Rockefeller African Dissertation Internship Award. Currently Mr. Rugutt is a candidate for the Doctor of Philosophy degree in Organic Chemistry (Natural Products). The Rockefeller Foundation have nominated Mr. Rugutt to receive the 1997-1998 African Science-Based Career Development Award (ASBD) to enable him continue with Striga research under the direction of Prof. Nikolaus Fischer and in collaboration with Dr. Dana K. Berner of the International Institute of Tropical Agriculture, Ibadan Nigeria.
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Joseph Kipronoh Rugutt
Major Field: Chemistry
Title of Dissertation: Control of African *Striga* Species by Natural Products from Native Plants

Approved:

[Signatures]

Major Professor and Chairman
Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

Date of Examination: April 8, 1996

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.