INSECTICIDAL COMPOUNDS FROM *AGLAIA* SPECIES

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ABSTRACT

In our continuing programme of screening Sri Lankan plants for insecticidal activity, plant extracts are screened at Peradeniya and at Basle, Switzerland and where activity is observed, bioactivity directed fractionation is undertaken to isolate the active compound.

Our work on *Aglaia apiocarpa*, a Meliaceae growing in the Knuckles range led to the isolation of a cyclopentanotetrahydrobenzofuran rocaglamide which showed high insecticidal and herbicidal activity. Studies on a more common species of *Aglaia, A.elaeagnidea* showed it to contain roclagamide as well as four other related compounds of high activity. These compounds have shown activity against a wide range of insects at low concentration and represent the best results of our screening programme so far.

Extracts from this plant could be used for pest control by Sri Lankan farmers and the activity of the compounds themselves indicate that they have the potential for commercial exploitation by the plant protection industry.

INTRODUCTION

The environmental hazards posed by the large scale use of synthetic insecticides have prompted the search for new and safer pest control agents. Botanical pesticides have been used by man from time immemorial. One of the early biopesticides used was the tobacco plant whose active ingredient, nicotine is extremely toxic providing evidence that not all biopesticides are environmentally acceptable. The success use of the relatively harmless pyrethroids from the flower head of the Kenyan *Chrysanthemum cinerariefolium* and more recently, Neem (*Azadirachta indica*) seeds in plant protection, has renewed interest in plants as a source of pesticides. We have been conducting a programme of screening Sri Lankan plants for pesticidal activity. Where activity is observed, we have attempted to isolate the compound(s) responsible for the activity. We now report the important results from this study and the isolation of a strongly insecticidal compound from *Aglaia elaeagnidea*.

MATERIALS AND METHODS

Extraction of plant material

Plant material usually stem bark, leaves, fruits, root bark are collected, dried, ground and successively extracted at 27° with dichloromethane and methanol for two twenty four hour periods. The solutions are then filtered and concentrated below 40° to give the dichloromethane and methanol extracts, which are screened for insecticidal activity at Peradeniya or Basel, Switzerland.

Screening for activity at Peradeniya

Aedes aegypti eggs were placed in a beaker containing water for 24 h. The eggs hatch in 24 h and after a further 24 h, the second instar larvae used in the screening was available.

Water (75 ml) and 45 μl of polyethyleneglycol (PEG) were added to the extract (30m g) in 0.5 ml of acetone and ten second instar larvae were introduced into 10 ml of this 400 ppm solution contained in a beaker. The concentration of the solution was decreased to 200, 100 and 50 ppm by serial dilution and all concentrations were tested with four replicates. Acetone and PEG in water made up in a similar manner but without extract was used as control. Mortality was observed after 24 h.

Where activity was observed more dilute solutions were tested to determine the minimum effective concentration.

Fractionation of extracts

Active extracts were fractionated using vacuum liquid chromatography and the fractions were screened for activity as above at concentrations of 200 ppm and below. Active fractions were subjected to repeated column, medium pressure liquid and flash chromatography with fractions being screening for activity at concentrations below 100 ppm until pure compounds of high activity were isolated.The active compounds were characterised using ultraviolet, infrared, nuclear and mass spectroscopy.

Screening for activity at Basel, Switzerland

Active extracts and compounds were screened against twenty organisms in a general screen at Basel, Switzerland. Compounds found to be strongly active were subjected to field trials at Basel.

RESULTS AND DISCUSSION

The compounds responsible for a variety of activities shown by a large number of plant extracts have been identified.

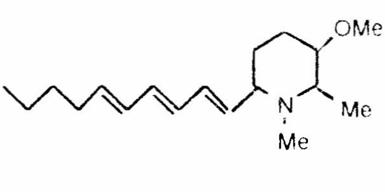
Bioactivity-directed fractionation of *Aegle marmelos* extract showed that dictamine and robustine were strongly active against *Aedes* larvae, while auroptene and O-methylhalfordinol were less active. Umbeliferone and epoxyauroptene showed growth inhibition. The mosquito larvicidal activity of *Persea gratissima* leaf extract was shown to be due to heptadec-16-ene-1,2,4-triol.

The mosquito larvicidally active material of *Clausena lansium* was found to be due to chalepsin and that of *Murraya koenigii* stem bark due to 2-methoxy-3-methyl-carbazole.

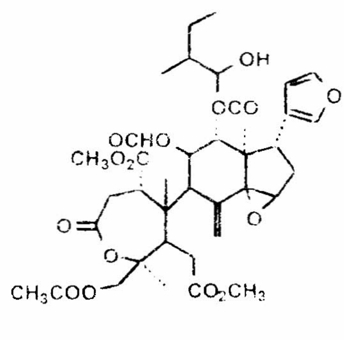
Bioactivity-directed fractionation of *Pamburus missionis* extract showed that its activity against the insects, *Heliothis* and *Diabrotica* was due to imperatorin and similarly, that the phytotoxic activity of *Michelia champaca* was due to parthenolide. The insecticidal activity of *Curcuma zedoaria* rhizome was shown to be due to the known furanodiene, (1E, 4E)-8,12-epoxygermacra-1(10),4,7,11-tetraene (Hikino *et al.* 1970) and that of *Zingiber purpureum* rhizome to the known 4-(3',4'-dimethoxyphenyl)but-1,3-diene (Masuda and Jitoe, 1995), both of which had previously been isolated from the same source.

The activity of *Microcos paniculata* was shown to be due to the alkaloid, N-methyl-6β-(deca-1',3',5'-trienyl)-3β-methoxy-2β-methylpiperidine (**1**). Although the piperidine has not been previously reported, a related compound has been isolated from *Microcos philippinensis* (Aguinaldo and Reed, 1990). The piperidine **1** was found to be strongly active against mosquito larvae, larvae of the diamond back moth - *Plutella xylostella* and the cowpea bruchid - *Callosobruchus maculatus* and its possible use in pest control is being evaluated in the field.

Among the most active compounds isolated in the project was the antifungal principle of *Pseudocarappa championi* and the insecticidal compounds of two *Aglaia* species. Although the activity of the antifungal compound, epoxypreurianin (**2**) (Connolly *et al.* 1979) against the grape fungus, *Botrytis cinerea* was at a level which could have been exploited commercially, the compound itself was present only in small amounts in the bark of a large Meliaceae perrenial and the structure of epoxypreurianin with seven asymmetric centre did not allow itself to be easily synthesised.



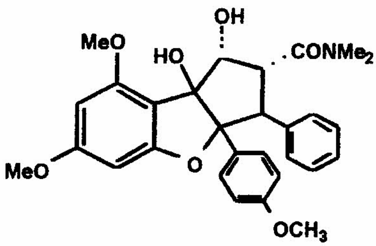
Piperidine (1)



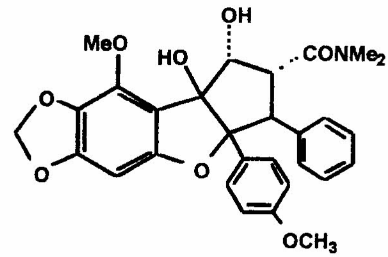
Epoxypreurianin (2)

Our studies on *Aglaia apiocarpa* (*A. congylos*), a Meliaceae tree growing in the Knuckles range led to the isolation from its bark, of a cyclopentatetrahydrobenzofuran, aglacongin (**3**) which showed high insecticidal and herbicidal activity. The compound although new at the time of isolation has been reported as rocaglamide (**3**) since then (Janprasert *et al.* 1993). Studies on the bark of a more common species of *Aglaia* growing in the Madugoda district, *A. elaeagnoidea*

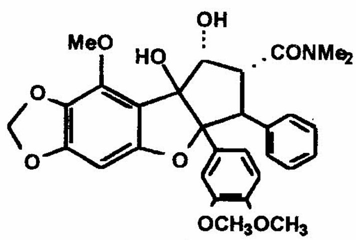
(*A. roxburghiana*) showed it also to be strongly active. Aglacongin was shown to be present in the extract. However, careful separation of the extract showed that there were at least four related compounds of high activity. The compounds which were present in



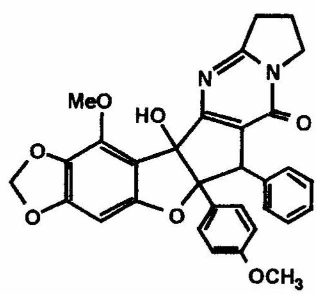
Roclagamide (3)



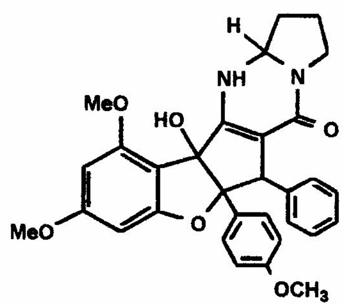
Aglaroxin A (4)



Aglaroxin B (5)



Aglaroxin C (6)



Aglaroxin D (7)

small quantities and overlapping in chromatographic analysis could only be separated by repeated medium pressure liquid chromatography followed by high performance liquid chromatography. They were new compounds and have been named aglaroxin A (**4**), aglaroxin B (**5**), aglaroxin C (**6**) and aglaroxin D (**7**) (Molleyres and Kumar, 1996). All of them showed strong activity against a wide range of insects and were effective both as contact poisons and systemic insecticides. They also showed acaricidal activity and could be used to control mites. An attempt is being made to synthesize these compounds in order to market them as crop protection agents.

We have initiated a project to study the seeds of the tree in an attempt to provide the Sri Lankan farmers with a renewable cheap and effective pest control agent.

CONCLUSIONS

Aglaia elaeagnidea stem bark has been shown to contain roclagamide and four related new cyclopentanotetrahydrobenzofurans - aglaroxin A, aglaroxin B, aglaroxin C and aglaroxin D. The four aglaroxins were sufficiently active at low concentrations against a wide range of insects and arachnids to be considered for commercial use by the plant protection industry.

ACKNOWLEDGEMENT

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REFERENCES

Aguinaldo, A. M. and Reed, R. W. (1990) A major piperidine alkaloid from *Microcos philippinensis*. *Phytochemistry*, **29**, 2309-2313.

Connolly, J. D., Labbe, C., Rycroft, D. S., Okorie, D. A. and Taylor, D. A. H. (1979) Tetranortriterpenoids and related compounds. Part 23. Complex tetranortriterpenoids from *Trichilia preuriana* and *Guarea thompsonii* (Meliaceae) and the hydrolysis products of Dregeanin, Prieurianin and related compounds. *J. Chem. Res. (S)*, 256-257.

Hikino, H., Agatsuma, A., Konno, C. and Takemoto, T. (1970) Sesquiterpenoids XXXV, Structure of furanodiene and isofuranogermacrene. *Chem. Pharm. Bull. (Japan)*, **18**, 752-755.

Janprasert, J., Satasook, C., Sukumalanand, P., Champagne, D. E., Isman, M. B., Wiriyachitra, P. and Towers, G. H. N. (1993) Rocaglamide, a new natural insecticide from *Aglaia odorata* (Lour.) (family Meliaceae). *Phytochemistry*, **32**, 67-69.

Masuda, T. and Jitoe, A. (1995) Phenylbutenoid monomers from the rhizomes of *Zingiber cassumunar*. *Phytochemistry*, **39**, 459-461.

Molleyres, L.-P. and Kumar, V. (1996) Insecticidal polycyclic compounds. Patent, W. I. P. O., International Publication No. WO 96/04284.