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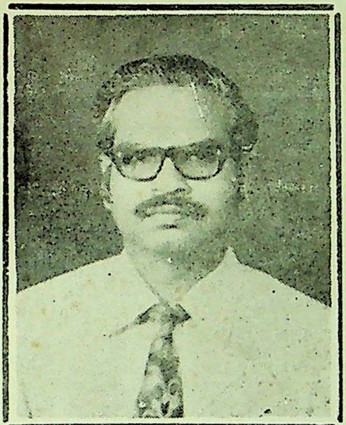
FACULTY OF SCIENCE
UNIVERSITY OF JAFFNA

Professor S. MAGESWARAN MEMORIAL LECTURE 1999



Insecticidal Compounds from Sri Lankan Plants

by

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INSECTICIDAL COMPOUNDS FROM SRILANKAN PLANTS

Professor S. Mageswaran Memorial Lecture

Vijaya Kumar

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Head of Department
Department of Chemistry,
University of Peradeniya,
Peradeniya.

Ladies and Gentleman,

It gives me great pleasure to present the first Professor S.Mageswaran memorial lecture. I would have liked very much to personally deliver this lecture today but unfortunately I am not able to free myself at this time to spend the few or perhaps several days in Jaffna that this may involve.

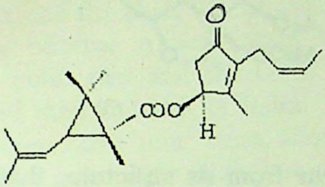
I firmly believe that Mageswaran was one of the finest synthetic organic chemists Sri Lanka ever produced. Much of the work on ylid chemistry which made Professor Ollis famous was carried out Mageswaran's laboratory bench at Sheffield. Mageswaran could not make use of his abilities in synthetic organic chemistry when he returned to Peradeniya, because although Peradeniya was the leading centre of organic chemistry research in Sri Lanka, it had very little facilities for synthetic work. If Mageswaran had remained at Peradeniya, he probably would have set up a vibrant synthetic chemistry group, but he decided instead to take up the challenge to develop the Department of Chemistry at Jaffna. If Jaffna had not been transformed into the focal point of this continuing thankless war, Mageswaran would have worked to set up that group in Jaffna. And he would have achieved it because he was one of the most reliable, dependable and hard working people I came to know. He had the initiative to see that a job was done and done well at that. His love for chemistry made him an excellent teacher, who worked hard at imparting his ideas and knowledge to his students. It was his leadership and dedication, which allowed the Chemistry Department in Jaffna to survive the ravages of war and produce such excellent graduates with a sound knowledge of Chemistry.

Today, I wish to present some of our work at Peradeniya in my lecture "Insecticidal Compounds from Sri Lankan Plants" as the Professor S.Mageswaran memorial lecture.

One of our projects at Peradeniya has been aimed at producing insecticidal compunds from Sri Lankan plants. Synthetic insecticides like the carbamates, organophosphates and organochlorine compounds as you all know pose environmental dangers to both the farmer and the consumer of his products. In Sri Lanka, for example, the use of pesticides is rarely monitored and very often farmers used much larger doses than those recommended and take very few precautions while using them. The risks involved in the use of synthetic insecticides have prompted the search for new and safer pest control agents.

Botanical pesticides or biopesticides have been used by man from time immemorial. Although they have been freely used by traditional farmers, biopesticides are not necessarily harmless and some of them may not be environmentally acceptable. For example, one of the early biopesticides used was the tobacco plant whose active ingredient, nicotine, as you all know is extremely toxic.

However, there has been a new interest in the study of plants as a source for pesticides. This is because of a few recent success stories in this field. The flower head of the Kenyan plant belonging to Family Compositae, *Chrysanthemum cinerariefolium* was found to contain the terpenoid compounds called the natural pyrethroids. Natural pyrethroids are esters of a cyclopropane carboxylic acid and a five membered ring alcohol, e.g. cinerin 1 (1). They were found to be effective insecticides with low human toxicity. Although the natural pyrethroids were not difficult to synthesize, they were found to be photolabile, i.e they were not very stable in sunlight. So they could not be used in the field. Elliot and his group at Rothamstead in the United Kingdom made several analogues of the natural pyrethroids and a few of them were found to be suitable for use as insecticides because they were found to be not only effective and harmless but also relatively stable. Because of their low toxicity, many synthetic pyrethroids are used in mosquito coils.



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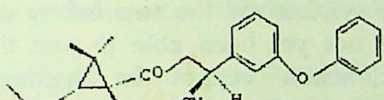

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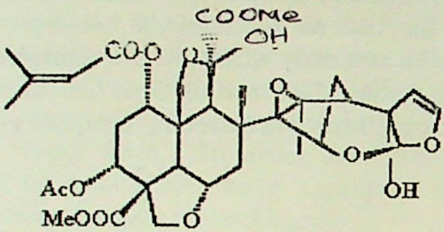
More recently, the Margosa, Neem or Vempu (*Azadirachta indica*) has been rediscovered by the West as a natural insecticide. Indian farmers had used water extracts from its leaf and seed for the protection of crops from insect attack. The seed shows a wide range of activities including activity against a wide range of insects, nematodes, arachnids, etc. The activity is due to the presence of a number of oxygenated terpenoids many of which contain a furan side chain. Azadirachtin (3) is an example of this class of compounds which are called limonoids and is the major limonoid constituent of the seed extract.



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It is clear from its structure, that Azadirachtin is not easy to synthesize. Ley and his group at Cambridge have synthesized the two halves of the molecule but have not yet been able to join them together. So, Azadirachtin cannot be synthesized for use in agriculture. Even if it can be synthesized, it may not be possible to make sufficient quantities for use because unlike drugs used in medicine, large quantities of material are required for use in pest control. Furthermore, it has been shown that the Neem extract is more active e.g. as a nematocide, than the individual compounds present in the extract.

Fortunately, for religio - cultural reasons, large areas of central India have been grown with Neem trees over the centuries and a ready source of the seed exists. Perhaps, at one time, North Sri Lanka was similarly full of Neem trees. Thousands of tons of Neem seeds can therefore be collected and extracted to give a seed extract which is then formulated into a commercial product. In fact it was an American company, Grace which first patented a Neem extract in the United States and obtained approval for its product, Magosan O for use as an insecticide in horticulture. Indian Scientists were shocked to hear that a preparation well known to the Indian farmer for centuries as an insecticide had been accepted by the U. S. Patent Office as an invention. What the company had claimed in the patent is that with their product, they were able to extend the shelf life of the Neem extract. However, this became a sore point among Indian Scientists and politicians and the U. S. Company has not tried to sell their product in India. In fact, many Indian companies have now been allowed to patent their preparations not only in India but also in the U. S, so that there are many Indian Neem products in the market. Some of these are sold in Sri Lanka as well.

Neem extracts have insect growth regulatory effects. They do not immediately kill an insect, but act on the life cycle of the insect producing deformed adults in the next generation or the one after. Neem extracts will therefore be less harmful to man and also the development of resistance to the insecticide by the insect will also be delayed. Although such insecticides are environmentally more acceptable, farmers in countries like Sri Lanka unfortunately prefer insecticides which are seen to kill insects immediately.

In our work at Peradeniya, we screen plant extracts for insecticidal activity and where activity is observed, we attempt to isolate the compound(s) responsible for the activity in the hope that we may be able to discover a compound like Pyrethrin which could be developed into a crop protection agent.

Although strongly insecticidal compounds may be isolated, they cannot often be used directly for a variety of reasons. As we saw in the case of Azadirachtin, some cannot be synthesized cheaply in large quantities, and as in the case of the natural Pyrethroids, some are photolabile and do not last long enough in the presence of light to affect the insect population. Sometimes the compound although a good insecticide, may have other properties like herbicidal activity which make them less attractive. So, very often, the active natural product itself cannot become a commercial product. As we saw in the case of the Pyrethroids the natural product serves as a lead compound which could be used as a guide to prepare related compounds. These compounds are tested for activity and stability and if a synthetic compound is found to show good activity, is stable, has no undesirable properties and can be synthesized cheaply, the synthetic analogue will have commercial potential.

The first step in our work is to collect the plant material. Sri Lanka as you know is a small island but within the boundaries of this small island, we have a variety of environments including the rain forests, the montane forests, the dry zones and the mangroves. In addition a quarter of the three thousand higher plants growing in Sri Lanka are endemic, i.e. growing only in Sri Lanka. So we have access to a large number of plants from many different environments, some of which are found nowhere else and have not been previously screened for activity. Unfortunately, the fauna of Jaffna and its surroundings have not been accessible to us or even to the Scientists of the University of Jaffna for several years now.

The plants themselves have to be selected from the thousands of plants available in Sri Lanka. One obvious method of selection is on the basis of folklore on their traditional use for pest control by farmers. Neem is one such plant whose study has led to a commercial product. Unlike in traditional medicine, the folklore on pesticides is limited to in fact only about five plants other from Neem. Of these, one we cannot identify what its botanical name is while another, ***Ocimum sanctum*** is thought to be mosquito repellent and has been well studied. Its extract, which contains only the non - volatile part is inactive. Perhaps the strong "eugenol" - type smell of the plant repels mosquitoes. The other two, ***Vitex negundo*** and ***Gnidia glauca*** show some weak activity. So, our work based on folklore knowledge has not been very successful. However, we have looked at some plants with folklore use in traditional medicine and found many of them show some pesticidal activity.

We have also been collecting plant material which is observed in the forest as being resistant to attack and also when literature reports suggest that certain plants are active, we have looked at related plants found in Sri Lanka. The latter has been the most successful strategy.

The parts of the plant studied are usually the stem bark, leaves and the root. If fruits and seeds are available, they are studied. If the root is available in large quantities we may separate the root bark from the root. The root bark usually has a high concentration of secondary metabolites. In the case of timber trees, we may also study the timber.

The plant part is collected, dried in the shade, ground in a mechanical grinder and extracted successively with dichloromethane and methanol in a shaker. Extraction is carried out at ambient temperature usually for two 24 hour periods with each solvent. The combined dichloromethane and combined methanol extracts are separately concentrated in a rotavapor. . The dichloromethane and methanol extracts thus obtained are screened for insecticidal activity at our insectary at the Department of Chemistry at Peradeniya. However establishing a routine bioassay screen is somewhat expensive and we carry out only two routine bioassays and two other bioassays at Peradeniya. We therefore collaborate with a multinational company called Novartis (formerly Ciba -

Geigy Ltd) based in Basel, Switzerland who carry out about twenty bioassays in their general screening programme.

Screening at Peradeniya involves two routine tests, the mosquito larvicidal test using *Aedes aegypti* mosquito larvae and the residual film test using adult bruchids (***Callosobruchus maculatus***). In the former, aqueous solutions of the extract, fraction or compound in different concentrations is introduced into a beaker containing five or ten second instar mosquito larvae in water. If mosquito eggs are introduced into water the second instar larvae appears in one or two days. The effect of the material on the mosquito larvae is studied, usually over 24 hours.

In the residual film test, adult bruchids are introduced into a vial in which a thin film of the extract/ fraction/ compound is formed. The film is formed by introducing an acetone solution containing a known amount of the material into the vial, rotating it slowly until all the acetone has volatilised off and then drying the vial ***in vacuo***. The effect of the material on the adult bruchids is studied over 24 and 48 hr periods.

The toxicity of the material on the organisms and any other effects like growth inhibition and growth deformation are studied in these two bioassays.

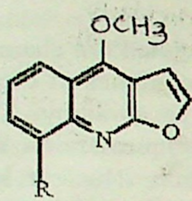
The other bioassays which we carry out involve the larvae of the diamond back moth, ***Plutella xylostella*** and the adult aphid, *Aphis craccivora*. A known volume of a solution of the extract on the organism itself can be applied using a microapplicator or the insects or larvae growing on host plant or plant part may be sprayed with a solution containing the extract using a Potter's Spray Tower or a rotating table. These bioassays will detect toxic effects, growth inhibition and other growth regulatory effects. There are also a number of other bioassays which could be carried out on these organisms and on the bruchid to determine anti - feedant activities, under choice, where organism can choose either treated or untreated material or no - choice conditions where it is left in the presence of treated material only.

If any extracts are active, we are interested in discovering the compound or compounds responsible for the activity. For this purpose we subject the extract to bioassay directed fractionation. The extract is fractionated using a series of chromatographic techniques like vacuum liquid, column, medium pressure liquid and flash chromatography and the fractions thus obtained are screened for activity using the same bioassays. Active fractions are subjected to repeated chromatography and the fractions obtained at each stage are again screened for activity. By this process what we are hoping to do is to obtain fractions with enriched activity and finally the pure active compound. Although theoretically one would expect it to be possible to isolate pure compounds of high activity by this method, this is not always possible. Sometimes the activity is often lost during the fractionation/ enrichment process. This can be due to decomposition of the active material during the fractionation process. Sometimes the activity is due to two or more compounds acting synergetically. i.e the activity of the compounds together is greater than that of them separately. If these compounds are separated into different fractions during fractionation, the activity of the different individual fractions will be less than that of original material. Since there are hundreds of compounds in the different fractions, it will be difficult to decide which of the fractions should be recombined to improve activity. Even so, we do occasionally isolate pure active compounds from active extracts.

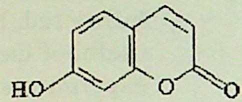
Active compounds which are isolated are characterised using standard spectroscopic techniques of Ultraviolet, Infra - red, Nuclear and Mass Spectroscopy. The most useful of the spectroscopic techniques are the different experiments in proton and carbon Nuclear Magnetic Resonance Spectroscopy, particularly the 2D-experiments. The active compound is often found to be a known compound. However, it is new compounds which are active, and particularly, such compounds with new modes of action which are interesting.

Active extracts and compounds also screened against twenty organisms in a general screen at Basel, Switzerland. The collaboration with Novartis helps to make sure that our extracts are subjected to a more comprehensive screen. At the same time, if we do find compounds of high activity, the high costs of development and protection of intellectual property rights make it almost impossible for us to develop the compound into a pesticide without the help of a company like Novartis.

Our screening results during the past ten years indicate that some families of plants show more activity than others. Generally extracts from members of the family Meliaceae to which Neem belongs and the related family Rutaceae, which includes the curry leaf plant, *Murraya koenigi*, have been found to show many different types of activities. We have also found that some parts of certain families show particular types of activity e.g. Clusiaceae barks are generally insecticidal.



(4) R=H (5) R=OH



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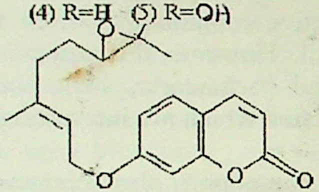

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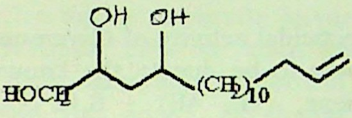
One of the first extracts which we subjected to bioactivity - directed fractionation was the extract of the root bark of *Aegle marmelos*. We showed that its activity was due mainly to the isoquinoline alkaloids, dictamnine (4) and robustine (5), which were active against *Aedes* larvae. Two coumarins present in the extracts, umbelliferone (6) and epoxyauroptene (7) inhibited the growth of the mosquito larvae. Although these compounds were active, there activities would not be considered good enough for further research and development into pesticides.

The mosquito larvicidal activity of *Persea gratissima* leaf extract was shown by one of Professor Mageswaran's students, presently on your staff, Dr Shanthini Saminathan who worked with us to be due to heptadec - 16 - ene - 1,2,4 - triol (8). Shanthini tried to make structural modifications on the triol in order to find out whether it would lead to enhanced activity, but triol (8) showed better activity than any of the derivatives prepared. Here too the activity although good was not enough for further work.



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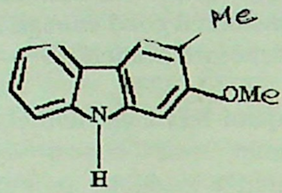
There were several other compounds which while active were not active enough. These included the carbazole alkaloid, 2 - methoxy - 3 - methylcarbazole (9) from ***Murraya koenigii*** stem bark and the coumarin, chalepensin (10) from *Clausena lansium* root bark, both of which were found to be the mosquito larvicidal. Bioactivity - directed fractionation of ***Pamburus missionis*** stem bark extract showed that its moderate activity against the insects, ***Heliothis*** and ***Diabrotica*** was also due to a coumarin, imperatorin

(11). Many furanocoumarins have been shown to have insecticidal activities.



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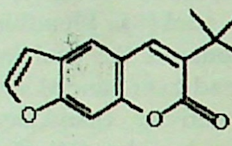

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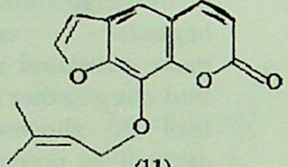

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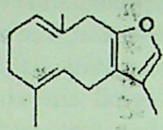
The insecticidal activity of ***Curcuma zedoaria*** rhizome was shown to be due to the known sesquiterpenoid, furanodiene, (1E, 4E) - 8,12 - epoxygermacra - 1(10),4,7,11 - tetraene (12) and that of ***Zingiber purpureum*** rhizome to the known aromatic diene, 4 - (3',4'- dimethoxyphenyl)but - 1,3 - diene (13), both of which had previously been isolated from their respective sources. The diene was found to be ovicidal to the bruchid, ***Callosobruchus maculatus***. Bruchid adults treated with the diene laid eggs, most of which did not develop embryos. The eggs appeared to be transparent unlike normal eggs. It is possible that the diene acts through a novel mode of action and we are making further studies on this activity.



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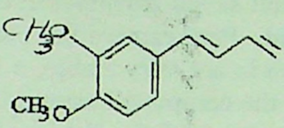

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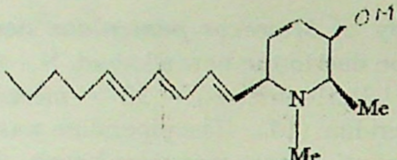
The activity of ***Microcos paniculata*** stem bark was shown to be due to the new alkaloid, N - methyl - 6β - (deca - 1',3',5'- trienyl) - 3β - methoxy - 2β - methylpiperidine (14). The piperidine was found to be strongly active against mosquito larvae, the larvae of ***Plutella xylostella*** and the adult cowpea bruchid, ***Callosobruchus maculatus***. Growth inhibition was observed and the adults developing from treated mosquito larvae and ***Plutella*** larvae and the larvae themselves were found to be deformed. The possible use of ***Microcos paniculata*** and the piperidine in pest control is being evaluated in the field by us, but preliminary studies suggest that the piperidine is photolabile.

Among the most active compounds isolated in the project, i.e. those with the potential of being developed into a commercial pesticide, was the antifungal principle of ***Pseudocarappa championii*** and the insecticidal compounds of two ***Aglaia*** species. Although the activity of the antifungal compound, the limonoid, epoxypreurianin (15) against the 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 endemic Meliaceae tree endemic to Sri Lanka. A close examination of the structure of epoxypreurianin shows us that seven asymmetric centres are present in the limonoid. Furthermore the compound has a epoxy group which is not very stable, a lactone group. This means that the compound cannot be easily synthesized. The development of the limonoid as a fungicide had therefore regretfully to be abandoned.



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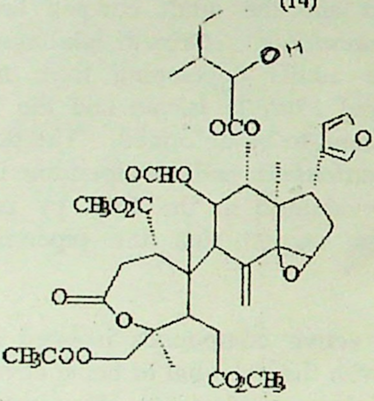

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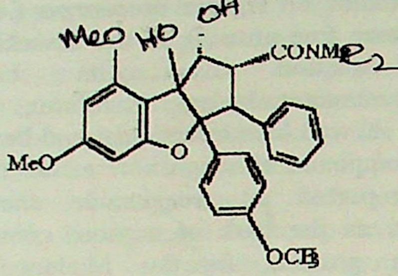
Our studies on *Aglaia apiocarpa* (*A. Congylos*), a Meliaceae tree growing in the Knuckles range led to the isolation from its bark, of a cyclopentanotetrahydro- benzofuran, aglacongin (16) which showed high insecticidal and herbicidal activity. The compound although new at the time of isolation was reported as rocaglamide shortly thereafter. Studies on the bark of a more common species of *Aglaia* growing in the Madugoda district, *A. elaeagnidea* (*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 twelve related compounds of high activity of which four, aglaroxin A (17), aglaroxin B (18), aglaroxin C (19) and aglaroxin D (20) are shown. The compounds were present in small quantities and since they overlapped in chromatographic analysis they could only be separated by repeated medium pressure liquid chromatography followed by high performance liquid chromatography. These four new compounds and their activities have been patented by us. They showed strong activity against a wide range of insects and were effective both as contact poisons and systemic insecticides. The development of these compounds into crop protection agents is still in progress.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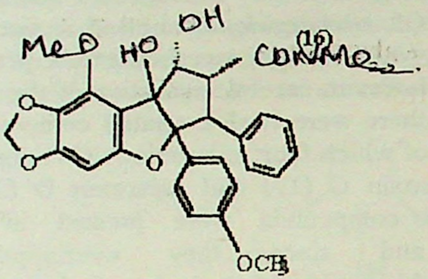
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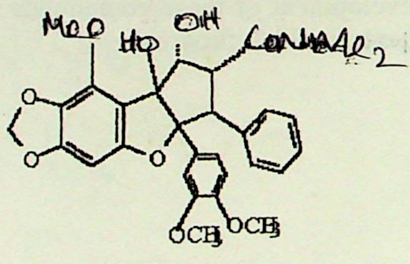
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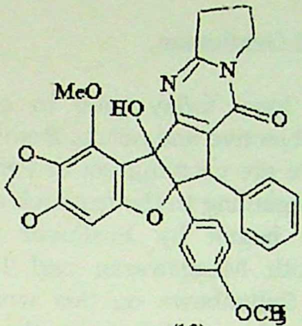

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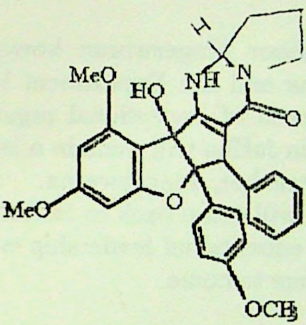

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Ladies and Gentleman,

I have today tried to convey to you the research objective and results from one of our research projects we are carrying out at Peradeniya. This work owes its beginning to the research on Natural Products Chemistry begun by Professor Sultanbawa in the 1970s. Both Mageswaran and I collaborated with Professor Sultanbawa on this work. So, in a sense, Mageswaran has contributed to the ideas that went into this work and had he remained in Peradeniya would no doubt have been an important member of our research group.

Professor Mageswaran however decided to move to Jaffna and the Department he built up here and the hundreds of exceptional organic chemists he has produced in Jaffna will remain a lasting monument to that great teacher, Mageswaran. I hope some of these students will come back to Jaffna and provide the scientific and educational leadership which Jaffna will need in the years to come.

Thank you for patiently listening to this lecture. I must once again apologise to you for my inability to deliver the lecture in person.

Vijaya Kumar, Senior Professor and Head of the Department of Chemistry, University of Peradeniya has been invited by the Professor S. Mageswaran Commemorative Committee to deliver the first Professor S. Mageswaran Memorial Lecture at the University of Jaffna.

Professor Kumar graduated with Honours from the then University of Ceylon in 1965 winning the Bhikaji Framjee Khan Gold Medal for the best performance in Chemistry in that year. He carried out his graduate work with Professor Sir Ewart Jones and Dr. G. D. Meakins at the Oxford University receiving his D. Phil in 1971.

He has been attached to the present University of Peradeniya since 1965 in various faculty positions culminating in the post of Professor of Chemistry in 1989.

Professor Kumar's choice of research fields, namely Insect Chemical Ecology, Development of New Insecticides from Natural Sources and Natural Products Chemistry, shows his zeal and willingness to use his knowledge and expertise in the cause of the Nation's scientific advancement.

Apart from more than 50 publications in International refereed Journals and over 90 communications to Learned Societies (National and International) Professor Kumar has been a major force in the systematization and dissemination of scientific knowledge in Sri Lanka. He has served and continues to serve on the Managing Boards of several scientific institutions. Professor Kumar also functioned as the Editor of the Ceylon Journal of Science (Physical Sciences) from 1991 - 1994 and has been a member of its Editorial Board since 1994.

Besides being the recipient of several Fellowships and Grants, Professor Kumar has been awarded many honours throughout his brilliant career. He received the Presidential Award for Scientific Achievement (Joint Awardee) in 1986 and in 1992 was elected a Fellow of the National Academy of Sciences, Sri Lanka.

Perhaps, Professor Kumar's most notable contribution to Chemistry in Sri Lanka is through his teaching undergraduates and postgraduates of the University of Peradeniya. In addition to teaching courses in all areas of Organic Chemistry at undergraduate level from 1966 to date Professor Kumar has supervised five Ph. D and eleven M. Phil students who have successfully defended their thesis at the University of Peradeniya.