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Trends in the Application of Natural Products in Plant Protection

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Insecticides of plant origin often provide models for the synthesis of more effective pest control agents with desirable properties. Recent advances in the area of insect endocrinology, behaviour and physiology have revealed that there are several chemical agents which control the insect growth, metamorphosis, behaviour and reproduction. Many such agents occur in plants and play important role in the intricate insect-plant interactions.

An extensive programme of screening the plant extracts and isolates for biological activities has been underway for sometime in the Bio-Organic Division, BARC. In this context, studies on insect-plant and insect-insect interactions are also underway. As a result of these studies, plant products showing moulting, juvenile and anti-juvenile hormonal, insecticidal and antifeedant activities have been isolated. Synthetic analogues of some active compounds have also been prepared.

The role of pheromones in the integrated pest management programmes has been well recognised in recent years. Successful syntheses of pheromones of insect pests of cotton, forest and orchard trees, potatoes, and stored grains, from inexpensive and readily available materials have been achieved.

A resume of investigation on natural products as well as synthetic work related to the insect control programme is presented.

Key Words: Natural Products, Pheromones

Introduction

As far as the world crop losses due to pests are concerned, it is estimated (Pimental & Pimental 1979) that nearly 1/3rd of the produce is destroyed by insects, pathogens, weeds, mammals and birds. The post-harvest losses are also very significant, which are estimated from 9% in the United States to 20% in some of the developing countries. Thus, nearly half of the world's food supply is being lost to the pests, who are consuming it or destroying it. This is an alarming situation.

The campaign to reduce crop destruction by pests has depended both on chemical and nonchemical (bioenvironmental) methods.

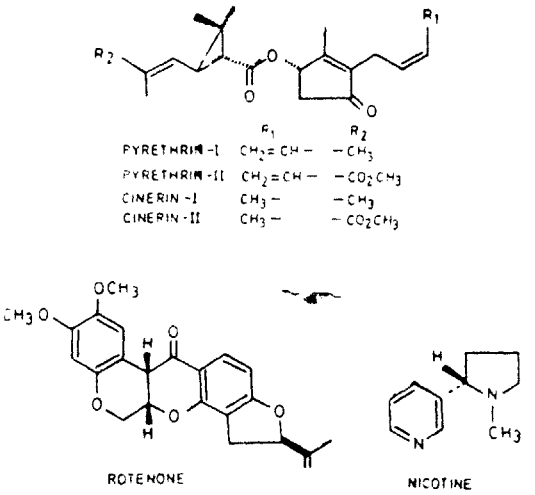
The extent of the use of chemical or non-chemical methods depends upon the nature of the malady viz., plant pathogens, weeds

or insects and also on the economic status of the country or the region. Of the more than 2 million tons of pesticides applied annually, nearly 1/3rd of the tonnage is used in North America, 1/2 in Western and Eastern Europe and Japan and the remaining (less than 20%) in developing countries. It is now well recognised that the pest control strategies, which rely heavily on pesticides, have a detrimental impact on the environment and the problems related to the development of resistance amongst insects are very well known. Such considerations have therefore given rise to an active search for new approaches to the pest control problems. The present communication describes the work being done at the Bhabha Atomic Research Centre (BARC).

These involve search for naturally occurring insect toxicants, insect growth regulators, insect antifeedants, and insect sex pheromones.

New Insecticides

In spite of the fact that a variety of secondary metabolites of plant and fungal origin afford protection against insects and fungi, only a relatively few of these, namely nicotine, rotenone and pyrethrins (figure 1) have been used in practice and have survived as commercial products. Nevertheless, the structures of natural pyrethrins have provided a basis for the development of a range of insecticides of great practical value by Elliott and his group (1978). Stepwise modification of the most potent of the pyrethrum esters viz., Pyrethrin I, has led to the synthesis of bioresmethrin, phenothrin, permethrin, decamethrin, cypermethrin, cyhalothrin, etc. (figure 2). The isosteric replacements involved in the modification of the model compound (pyrethrin-I) eliminated centres of photoinstability. Some of the synthetic pyrethroids, e.g. decamethrin, and the related dichloro- and chloro-trifluoromethyl



SOME NATURALLY OCCURRING INSECTICIDES

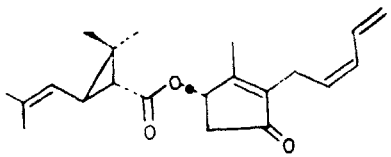
Figure 1

derivative have unprecedented insecticidal activity. This has rendered compound like decamethrin effective for field applications @ 7–50g/ha which is very low compared to the conventional insecticides.

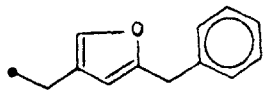
It is likely that systematic survey of plant materials, with the broad range of bioassays using different test species would lead to the discovery of new active compounds. Even though one may not always come across compounds possessing toxicity against pest insects comparable to the very potent currently available insecticides, it is likely that the structures of even moderately active compounds will provide useful leads to modify them into compounds with desirable activities against insect pests and relatively safe towards mammals and beneficial insects. There is a need for continuous search for new compounds with desirable properties.

Work being done at BARC

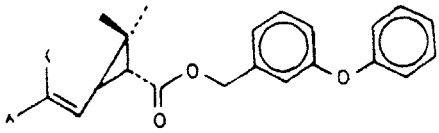
A systematic search at BARC for plant extracts for their insecticidal action revealed that garlic oil showed strong activity when tested against mosquito larvae. (Amonkar



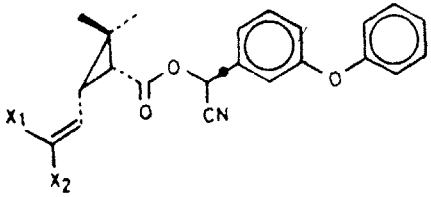
PYRETHRIN-I



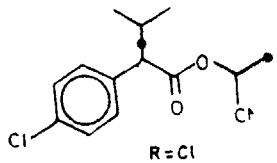
BIORESMETHRIN



PHENOTHRIN X=Me
PERMETHRIN X=Cl

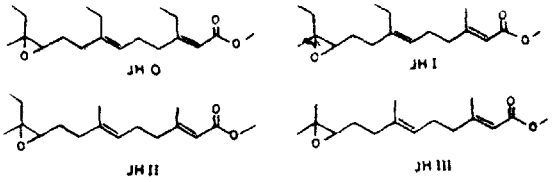


DECAMETHRIN $X_1 = X_2 = Br$
CYPERMETHRIN $X_1 = X_2 = Cl$
CYHALOTHRIN $X_1 = Cl, X_2 = CF_3$



R=Cl
FENVALERTE
PYRETHROID INSECTICIDES

Figure 2



STRUCTURES OF INSECT JUVENILE HORMONES

Figure 3

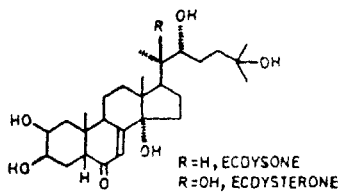
& Banerji 1971). It also showed good knockdown activity against adults. The active principle named 'Allitin' was shown to be a mixture of diallyl di-and tri-sulphides. A process for the large scale preparation was developed and sufficient quantities of allitin were prepared and supplied for field trials to a number of institutions. A radio-synthesis of allitin was developed and using tracer techniques, persistence of allitin and related compounds in insect and plant systems was studied. The metabolic fate of (^{35}S)-diallyl disulphide in mice showed that 70% of the radioactivity is present in liver cytosol, of which 80% is metabolised to sulphate. (Pushpendran et al. 1980). Investigations on mode of action of allitin showed that it acts by inhibiting the cholinesterase activity. (Bhatnagar-Thomas & Pal 1974). The unpleasant odour and low persistence of allitin has restricted its use as a general insecticide.

Piper cubeba extracts cause mortality to the last instar larvae of mosquito, *Culex fatigans*, and also show synergistic activity similar to piperonyl butoxide towards mosquito larvae when tested together with carbaryl and pyrethrin (Mulchandani et al., unpublished work).

Hormonal Insect Control

Insect Juvenile Hormones

Though the chemistry of the brain hormone is still elusive but the four juvenile hormones (figure 3) (JH-O, JH-I, II and III) and the moulting hormones (figure 4) stand well characterised. The pest control potential of IGR



R=H, ECDYSONE
R=OH, ECDYSTERONE

MOULTING HORMONES

Figure 4

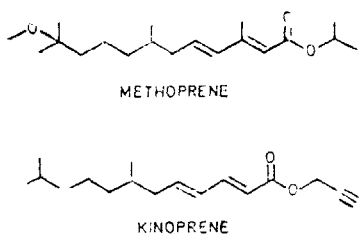


Figure 5

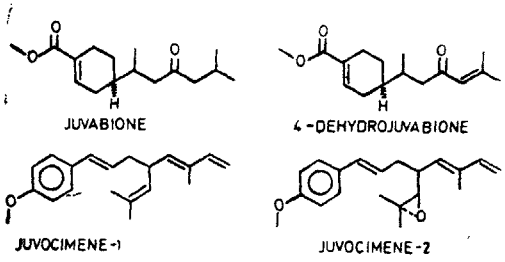


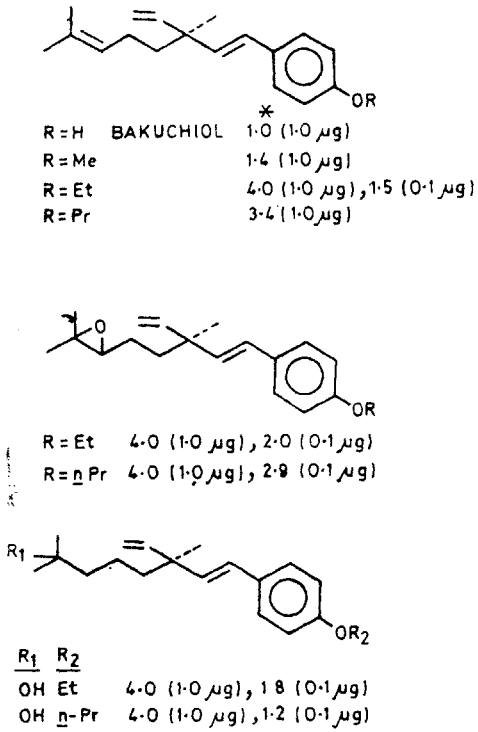
Figure 6

and promising JH mimics has been discussed by Dr Sukh Dev (in this issue) (figures 5, 6).

A survey of indigenous plants (Joshi et al. 1974) revealed that extracts of the common weed *Psoralea corylifolia* possess insect JH activitiy. Systematic fractionation of the extract followed by bioassay of each fraction led to the discovery that the active principle was bakuchiol, a compound isolated and characterised earlier by Sukh Dev and co-workers. Derivatisation of bakuchiol by Josh et al. (1979) resulted in a more active compound namely bakuchiol ethyl ether. Later (Bhan et al. 1980) made several derivatives which were initially tested by us when it was found that the 6, 7-epoxy propyl ether of bakuchiol was the most active compounds of the series (figure 7).

Insect Anti-juvenile Hormones

In 1976, Bowers announced the discovery of two compounds in the common bedding plant *Ageratum houstanianum*, which on contact with certain insects induced their precocious metamorphosis into tiny sterile insects. The effect was termed anti-juvenile hormonal effect which could be reversed by

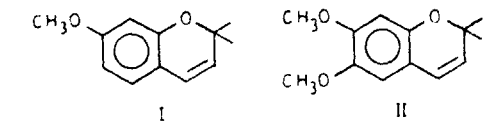


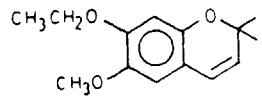
*These numbers refer to JH activity score. Score of 4.0 being highest Bokuchiol analogs (JH activities)

Figure 7

treatment of JH-III. The compounds were shown to be chromene derivatives named as Precocene I & II (figure 8) (Bowers et al. 1976). Optimization of the action of the precocenes through synthesis of analogues gave a variety of compounds more active than the two precocenes (Bowers 1977).

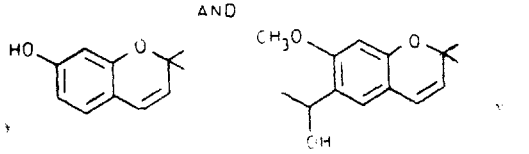
In addition to Precocene I & II, the related phenolic chromene and a hydroxyethyl chromene could be isolated from *Ageratum conyzoides* (Vyas & Mulchandani 1984) (figure 8A). It was also shown that precocenes arise in the plant from acetate and mevalonic acid (Vyas & Mulchandani 1980). A novel method for the syntheses of 6,7-substituted dimethyl chromenes was developed to study structure-activity relationship of anti-JH compounds. Radiosynthesis of precocene I & II (H^3-labelled) was also developed for metabolic studies (Banerji & Goomer 1981 a,b).





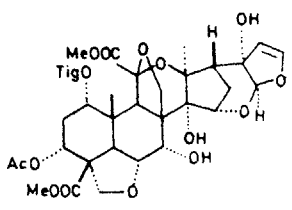
SOME ANTI-JUVENILE HORMONAL COMPOUNDS

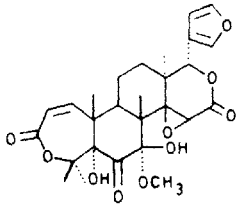
Figure 8

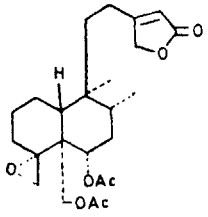


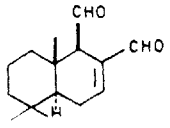
CHROMENES ISOLATED FROM AGERATUM CONYZOIDES

Figure 8A









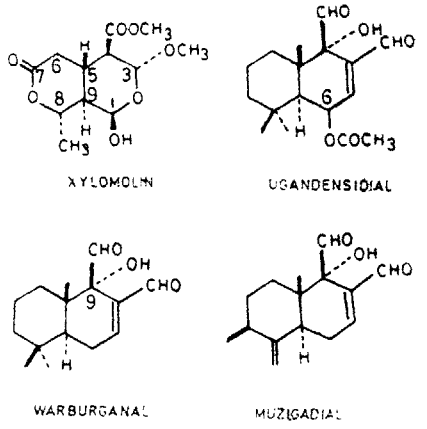
NATURALLY OCCURRING ANTIFEEDANTS

Figure 9

Insect Antifeedants

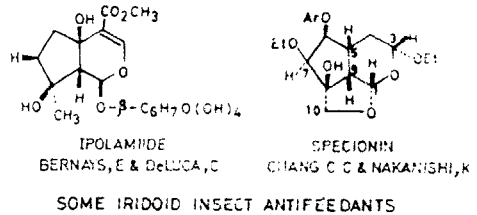
Use of neem (*Azadirachta indica*) leaves as natural repellent has been known for centuries in India. Although extensive work has been carried out in India on the bitter principles of neem oil, yet the effect of neem leaf extract on morphological changes in coffee bug, *Antestiopsis*, was observed in Kenya and efforts to isolate the active principle led to the isolation of azadirachtin, which was shown to act as a powerful feeding deterrent to locusts and also as a systemic growth disrupter. The structure of this amorphous and complex triterpenoid (figure 9) was established by Nakanishi and co-workers (Zanno et al. 1975, Kubo et al. 1976, Kubo et al. 1976). A systematic search for other insect antifeedants at International Centre for Insect Physiology and Ecology, Nairobi, led to the recognition of a number of active compounds (Meinwald et al. 1978). From *Ajuga remota* (Labiateae), moderately active antifeedants, ajugarin I, II and III were reported. While from the East African shrub *Harrisonia abyssinica*, a new antifeedant named harrisonin was isolated and characterised (figure 9). The other antifeedants isolated from East African plants were xylomolin from *Xylocarpus* sp. (Meliaceae), and polygodial and ugandensidial and previously unreported sesquiterpenoids, warburganal and muzigadial, from *Warburgia stuhlmannii* and *W. ugandensis* (figure 10).

The antifeedant properties of ipolamiidean iridoid antifeedant have been reported. (Ascher et al. 1981). Likewise, Cheng and Nakanishi have also reported the insect antifeedant activity of specionin which also is an iridoid insect antifeedant. Antifeedant activity of the diterpenes kalmitoxin-I and kalmitoxin-IV and grayanotin-III, isolated from *Kalmia latifolia*, against Gypsy moth has been reported by El-Naggar and his co-workers (figure 11).

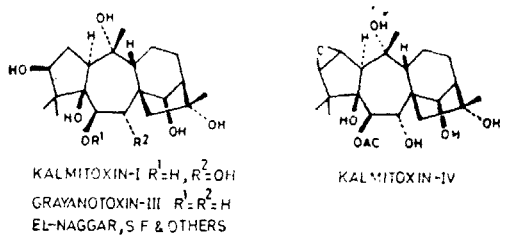


SOME MORE ANTIFEEDANTS FROM EAST AFRICAN PLANTS

Figure 10

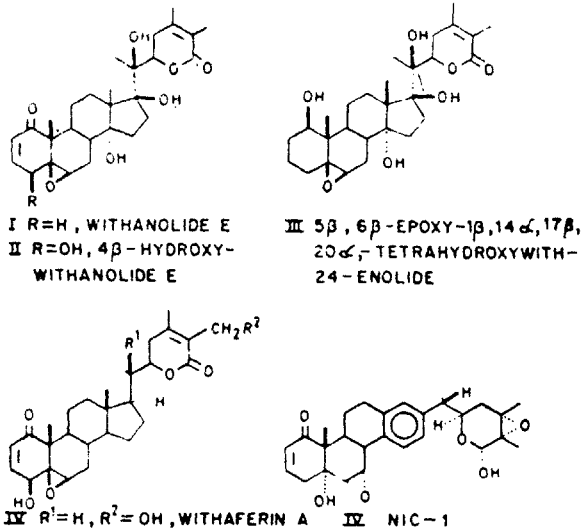


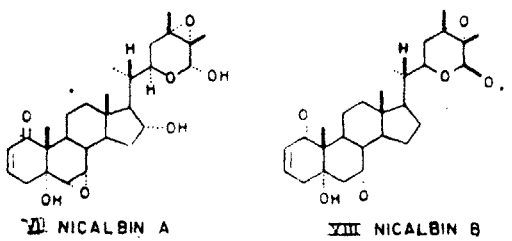
SOME IRIDOID INSECT ANTIFEEDANTS



SOME ANTIFEEDANTS DITERPENES

Figure 11

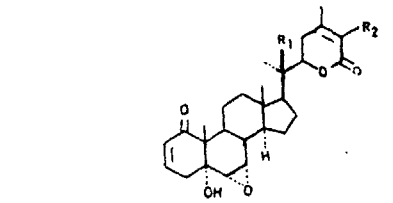




WITHANOLIDES AND RELATED ERGOSTANE TYPE STEROIDS AS ANTIFEEDANTS

Figure 12

Insect antifeedant properties of withanolides which were isolated from several solanaceous plants (Physalis, Withania and Nicandra species), have been discussed by Ascher et al. (1981). They tested eight structurally related withanolides (figure 12) and ergostane-type steroids and made some interesting observations. They divided the compounds into three groups and concluded that for the first group, the order of activity was I > III > II in *S. littoralis* and III > II > I in *E. varivestis*. In the second group, IV (withaferin A) and V (withanolide D) were inactive against both *S. littoralis* (at dose of 0.01%) and *E. varivestis* (at conc. of 0.1%). In the third group, however, NIC-I (VI) was inactive in *Spodoptera* at 0.01% and slightly active at 0.1%, whereas it was active even at 0.025% in *E. varivestis*. Furthermore, Nicabalin A (VII) and B (VIII) had both been inactive at 0.01% screening concn. in *S. littoralis* wherein Nicabalin A was highly active for *E. varivestis*. The selective activity against pest species may provide a useful tool to permit selective control of pest species.



WITHANOLIDES FROM WITHANIA SOMNIFERA

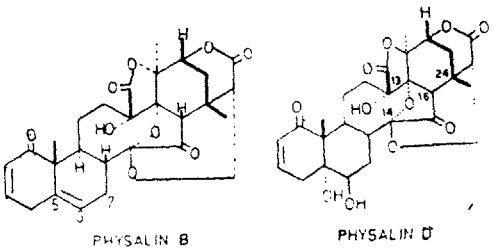
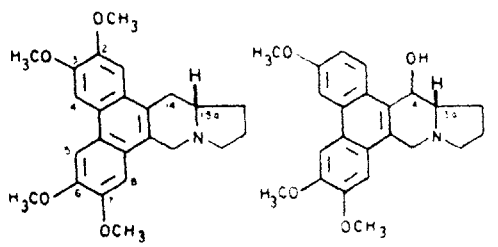
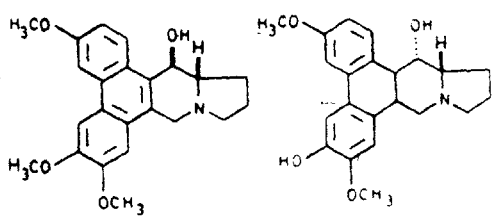


Figure 13 Physalins from *Physalis minima*





PHENANTHROINDOLIZIDINE ALKALOIDS FROM T ASTHMATICA

Figure 14

In connection with studies on the active principles of plant origin, a number of withanolides, viz. WS-I, WS-II, WS-III have been isolated and characterised (Mulchandani & Badheka, unpublished work). Besides Physalin B and D (Mulchandani et al. 1979) (figure 13) (table 1) which show antifeedant activity when tested against *Spodoptera litura*—the activity is, however, much weaker as compared with the antifeedant activity of azadirachtin (table 1). Screening of other plant extracts and their constituent principles revealed that the total alkaloid extract from *Tylophora asthmatica* exhibited powerful antifeedant activity when incorporated in a semisynthetic diet and tested against *Spodoptera litura* larvae. From the *T. asthmatica* total alkaloid extract, seven active principles were isolated, three of which were the known alkaloids, pergularinine, tylophorine and tylophorinine (figure 14). The antifeedant activity of the seven components (I to VII) are shown in (table 2)(Verma et al. 1986). Preliminary laboratory evaluation revealed that the antifeedant activity of tylophorine was comparable with that of azadirachtin, which was used as standard (table 3). This to our knowledge, is the first report of antifeedant activity of an alkaloid comparable to the activity of azadirachtin. Structure of components (IV to VII) *vis-a-vis* the antifeedant activity are being determined.

Thus, it will be observed that natural products of diverse structures can act as feeding-deterrents to insects. Studies on insect antifeedants of plant origin, therefore, offer immense potential to investigate insect-plant interactions and their role in evolutionary biology.

Insect Pheromones

In integrated pest management propagated today, the use of pheromones and other behavioural chemicals is assuming increasing

Table 1 *Antifeedant activity of some natural products against* Spodoptera litura *larvae*

Name of compounds	Conc.* (%)	Percent feeding inhibition over control
Azadirachtin	0.01	96.0
WS-I	0.1	44.1
WS-II	0.1	30.0
Physalin B	0.1	23.7
Physalin D	0.1	38.4

*Incorporated in Styrofoam

Table 2 *Antifeedant activity of* Tylophora asthmatica *compounds against* Spodoptera litura *F. larvae*

Compound	ID_{50} (ppm of the diet)*	Relative activity
I (Pergularinine)	12	7.25
II (Tylophorine)	3	29.00
III (Tylophorinine)	9	9.87
IV Unidentified	3	29.00
V -do-	3	29.00
VI -do-	84	1.03
VII -do-	87	1

Table 3 *Antifeedant activity of Tylophorine and azadirachtin against* S. litura *F. larvae*

Compound	Conc.* (%)	percent feeding inhibition over control
Azadirachtin (Standard)	0.001	58.33
Tylophorine	0.001	52.96

*Incorporated in cellulose diet

importance. Pheromones have found following applications:

(*i*) in baited traps for monitoring and surveying the area and the extent of infestation,

(*ii*) for luring the insect pests to the areas that are treated with pesticides or some subtle control agents, e.g. pathogens, chemosterilants, or juvenile hormones,

(*iii*) for mass trapping to achieve direct suppression of population, and

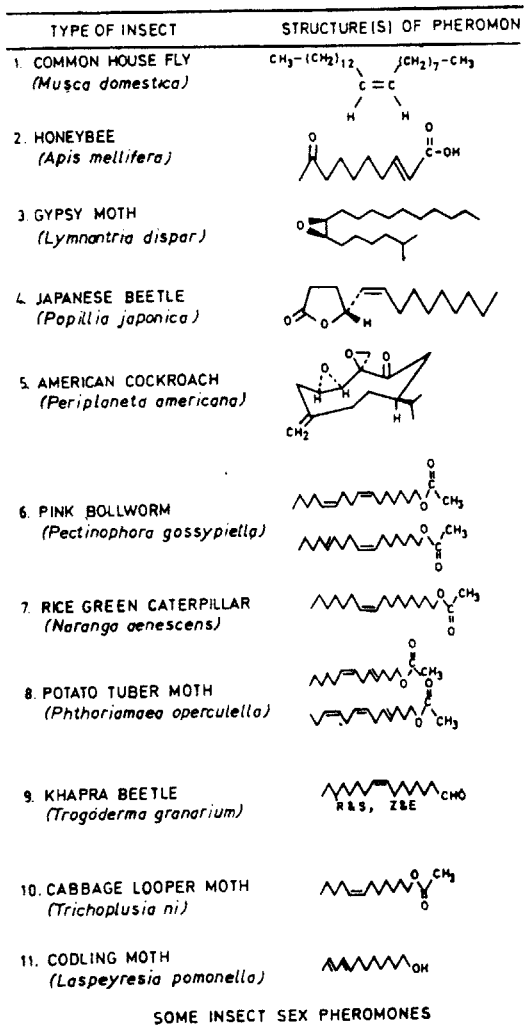


Figure 15

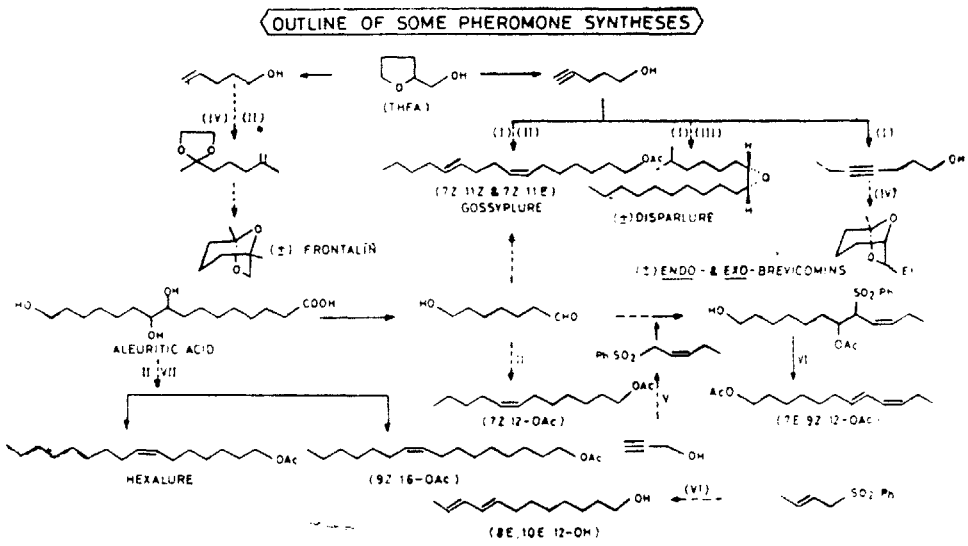


Figure 16

(*iv*) for permeation of infested areas with high concentration of synthetic pheromones, to cause confusion.

At present, the first application is being successfully exploited, whereas the other techniques are at various stages of development.

Of the very large number of the pheromones which have been characterised some of the important ones are shown in figure 15.

It can be surmised that synthetic chemistry has a pivotal role to play in pheromone research. Not only is an unambiguous synthesis necessary for the confirmation of a proposed structure, but also in many cases it has proved to be essential for the elucidation of correct structural and stereochemical features. Furthermore, since the quantities of pheromones available from natural sources are exceedingly small, it is only through chemical synthesis that enough material can be procured to make their application feasible.

Insect sex pheromones of agricultural pests such as pink bollworm moth, *Pectinophora gosypiella*, rice green caterpillar, *Naranga aenescens*, cabbage looper, *Trichoplusiani* have been synthesised (Joshi et al. 1984, Chattopadhyay et. al. 1983, Chattopadhyay et al. 1984). A practicalsynthesis of hexalure, a synthetic sex attractant for the male pink bollworm moth has also been achieved Chattopadhyay et al. 1984), The sex pheromones of codling moth, *Laspeyresia pomonella*, grape-vine moth, *Lobesia potrane*, peach twig borer, *Anarsia lineatella* have been synthesised (Kulkarni et al. 1983, Chattopadhyay et at. 1983). Syntheses of brevicomins and frontalin, the pheromone components of bark beetles, *Dendroctonus* sp and disparlure, the pheromone of gypsy moth, *Lymantria dispar*, the pests of forest trees, have also been accomplished (Joshi et al. 1983, 1984).

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