CHAPTER I

INTRODUCTION

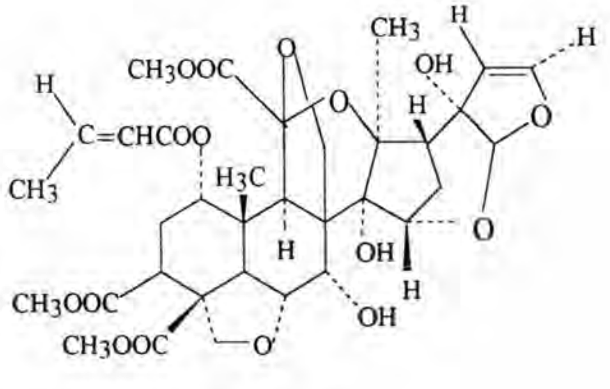
1.1 Insect Antifeedant

Insect antifeedant compounds are defined as chemicals that inhibit feeding, although they do not directly kill insects. They are insect antifeeding behavioural chemicals and are found in several plant parts. It is well known that special plant constituents function to establish interrelationships between insects and their food plants. An insect reaching or alighting on a plant is stimulated by attractants contained in the plant and inhibited by repellents. On the other hand, feeding stimulants elicit a feeding response of insects, whereas insect feeding is inhibited by the presence of feeding deterrents or antifeedants in plants (Natori *et al.*,1981). An intrinsic advantage of antifeedants is that by their nature they prevent damage, instead of killing the insect after it has damaged the plant as in the case of ordinary insecticides.

At present, environmental and health problems related to the use of synthetic pesticides have made more urgent the need for less toxic, environmentall friendly insect control substance. As part of this effort, a serious investigation for naturally occurring substance that posses insect antifeedant has been conducted. In the latter case, relatively few studies have been conducted with the aim of finding efficient compcunds with a potential for control.

Continuously, heavy usage of some chemical insecticides has created serious problems, the most obvious being direct toxicity to nontarget organisms such as when used for insect pest management, insect antifeedant might be especially advantageous because they control the insects indirectly through starvation, and they may not be harmful to parasites, predators, and pollinators. If crops were sprayed with efficient antifeedants, perhaps the pests would turn away from the crops (Munakata, 1977). If strongly active antifeedant can be developed, they should be valuable for crop protection from insects. These antifeedants have modes of action different from those of pesticides, and may overcome the environmental problems caused by pesticides.

Among the most potent classes of insect antifeedants, Azadirachtin **(1)** isolated from the seeds of Indian neem tree, *Azadirachta indica*, A.Juss, has been described by Zanno *et al.* (1975) and Warthen (1979). Its biochemical mode of action is however not fully understood (Mordue (Luntz) and Blackwell, 1993). Azadirachtin is known to have a profound effect on the feeding behavioural and neurophysiological studies have shown that azadirachtin stimulates receptors on neurones in the gustatory sensilla on the mouthparts of the lepidopteran larvae, its strong antifeedant. Antifeedancy varies markedly between species with Lepidoptera being particularly sensitive to azadirachtin. Feeding behaviour depends upon both neural input from the insect's chemical senses, with contact chemoreceptors on the tarsi, mouthparts and oral cavity being those mainly involved, and central nervous integration of this "sensory code". Despite the enormous volume of literature on chemoreception in insects and the sensory coding underlying the neural mechanisms leading to food selection behaviour. When azadirachtin is injected into insects it can cause a decrease in feeding over a 2-48 h peroid (Simmonds and Blaney, 1984).



Azadirachtin **(1)**

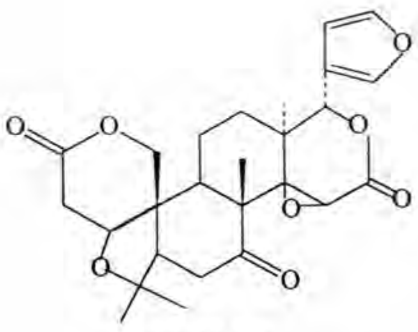
Furthermore, there are several classes of insect antifeedant such as limonoid, sesquiterpene, diterpene, quassinoid and cucurbitacin (Table 1.1). Nowadays, searching for insect antifeedant compounds is more interesting more than in the past with an aim to take these compounds for commercial application, to reduce the cost of production and to utilize conveniently.

Table 1.1 Examples of some antifeedants isolated from plants

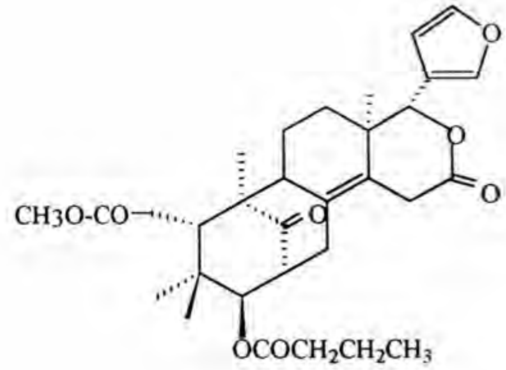
Type of compounds	**Antifeedants**	**Against insect**	**References**
1. Limonoid **(Fig. 1.1)**	limonin **(2)**	*Choristoneura fumiferana*	Alford and Bently (1986)
	methyl-3β-*n*-butyryloxo melic-8(14)-enate **(3)**	*Agrotis segetum*	Vanucci *et al.*(1992)
	deacetylgedunin **(4)**	*Reticulitermes speratus* Kolbe	Ishida *et al.* (1992)
	azedarachin C **(5)**	*Spodoptera exigua* Hubner(Boisduval)	Huang *et al.* (a) (1995)
	meliacarpinins 1 and 2 **(6,7)**	*Spodoptera eridamia* (Boisduval)	Huang *et al.* (b) (1995)
2. Sesquiterpene **(Fig. 1.2)**	angulatueoid G and H **(8,9)**	*Aulacophora femoralis* and *Piutella xylostella*	Dagang *et al.*(1992)
	argophyllin A and B **(10,11)**	*Diabrotica virgifera*	Alfatafta and Mullin (1992)
	8α-angeloyloxycostunolide **(12)**	*Spodoptera littoralis*	Goeren *et al.* (1994)
	polygodial **(13)** and 9-de-oxymuzigadial **(14)**	*Tineola bisselliella* and *Anthrenocerus australis*	Gerard *et al.* (1993)
3. Diterpene **(Fig. 1.3)**	scutegalin A and B **(15,16)**	*Spodoptera littoralis*	Rodriguez (1993)
	diterpene A,B **(17,18)**	*Tenebrio molitor* L.	Sosa *et al.*(1994)
	kaempferol 3-*O*-β-gluco pyranoside **(19)**	*Reticulitermes speratus*	Lajide *et al.*(a) (1995)
	kaur-16-en-19-oic acid **(20)**	*Reticulitermes speratus*	Lajide *et al.*(b)(1995)
	ajugarin I-II **(21,22)**	*Spodoptera littoralis*	Camps and Coll (1993) and Malakov *et al.* (1994)

Table 1.1 (cont.)

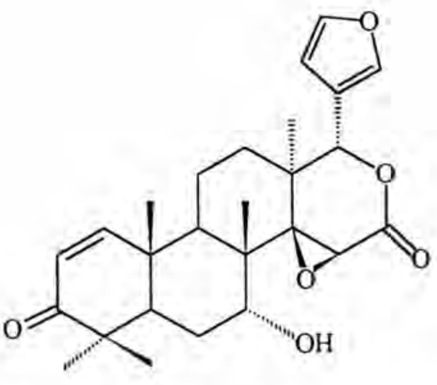
Type of compounds	**Antifeedants**	**Against insect**	**References**
4. Quassinoid **(Fig. 1.4)**	quassin **(23)**	*Plutella xylostella*	Daido *et al.* (1993)
	isobrucein-B **(24)**	*Plutella xylostella*	Daido *et al.* (1995)
	guineensino **(25)** pipercide **(26)** and chingchengenmide A **(27)**	*Plutella xylostella* and *Aedes aegypti*	Montein-Art (1995)
5. Cucurbitacin **(Fig. 1.5)**	2-*O*-β-D- glucopyranosyl-cucurbitacin E **(28)** and 2-*O* -β-D-glucopyranosylcucurbitacin I **(29)**	*Pieris rapae*	Gupta *et al.* (1993)



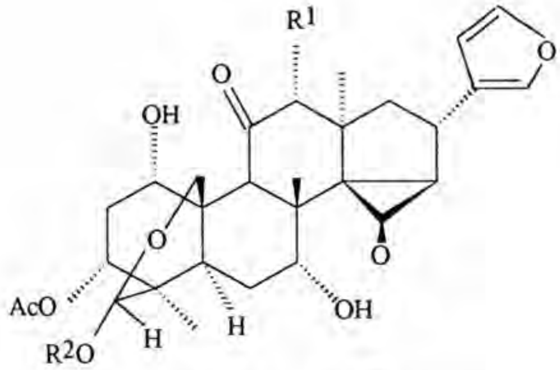
limonin **(2)**



methyl-3 β-*n*-butyryloxy-1-oxomeliac-8(14)-enate **(3)**

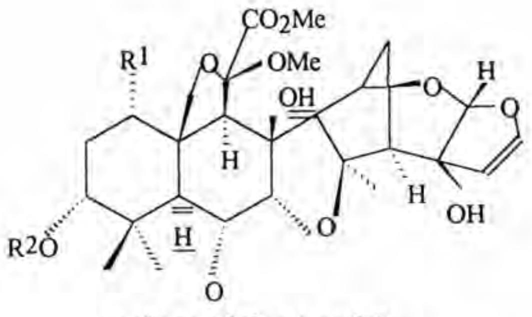


deacetylgedunin **(4)**



azedarachin C **(5)**

R^1 = H ; R^2 = COCHMe$_2$



meliacarpinins 1 and 2

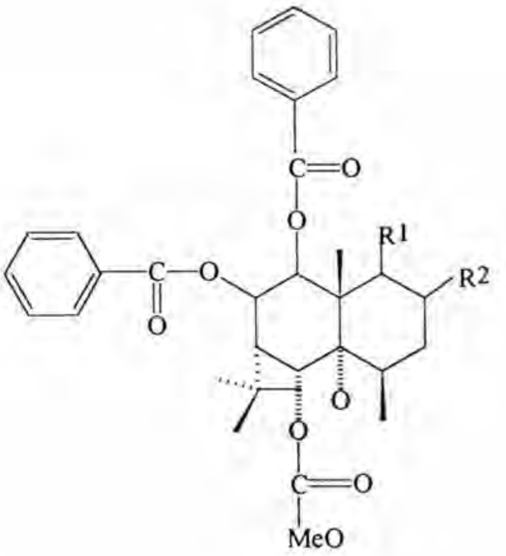
R^1 = OCinn ; R^2 = Ac **(6)**

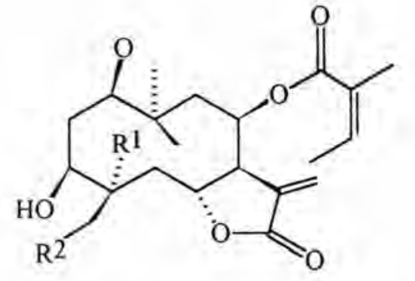
R^1 = H ; R^2 = Tig (7)

Fig. 1.1 Limonoid class antifeedants angulatueoid G and H

R^1 = OH ; R^2 = OH **(8)**

R^1 = OAc ; R^2 =H **(9)**

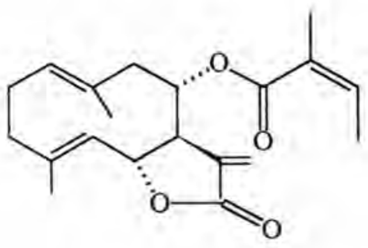




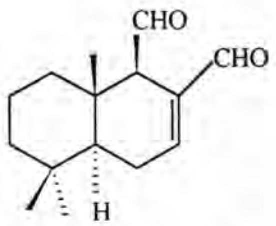
argophyllin A and B

R^1 = OH ; R^2 = H **(10)**

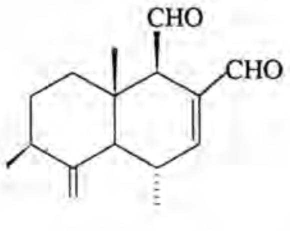
R^1 = H ; R^2 = OH **(11)**



8 α- angeloyloxycostunolide **(12)**

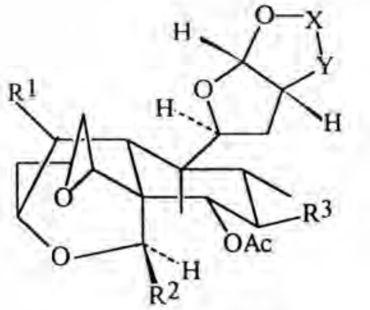


polygodial **(13)**



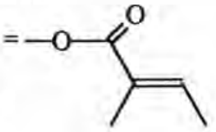
9-deoxymuzigadial **(14)**

Fig. 1.2 Sesquiterpene class antifeedants

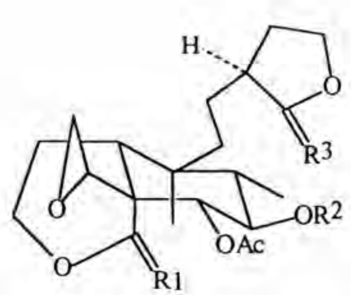


scutegalin A **(15)**

$R^1 = H$, R^2 , R^3 =

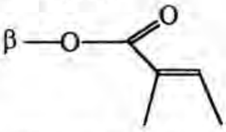


X-Y = $H_2C\text{-}CH_2$

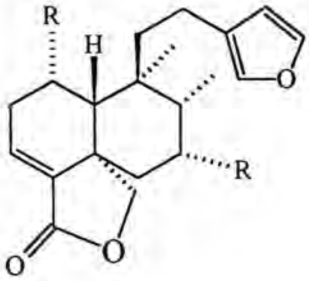


scutegalin B **(16)**

R1 = α H,β

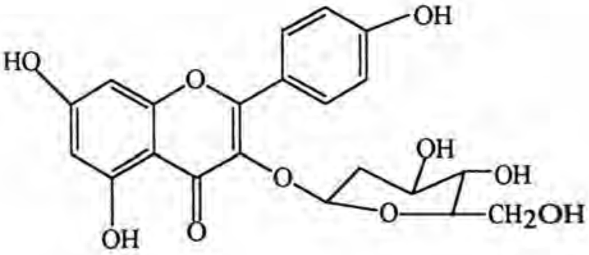


$R^2 = H$, $R^3 = H.\ OH$ (R and S)

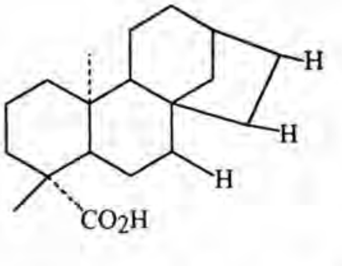


R = H **(17)**

R = OH **(18)**

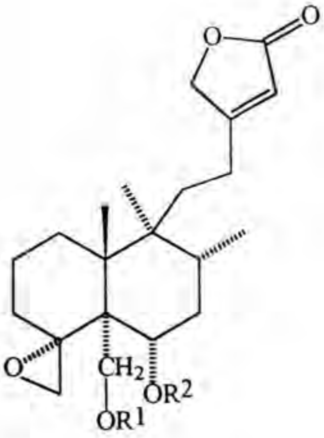


kaempferol -3-*O*-β-glucopyranoside **(19)**



kaur-16-en-19-oic acid **(20)**

Fig 1.3 Diterpene class antifeedants

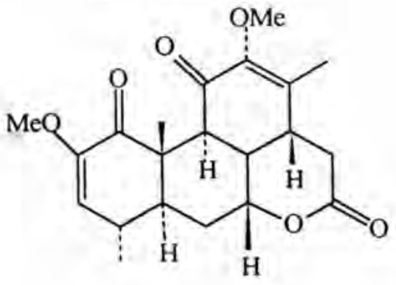


ajugarin I and II

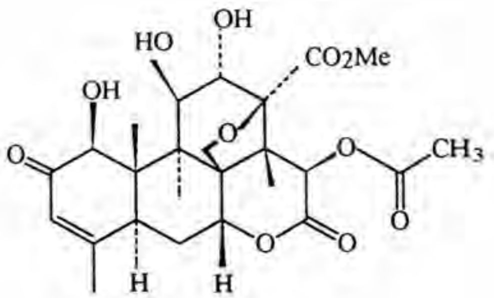
R1 = Ac ; R2 = Ac **(21)**

R1 = Ac ; R2 = H **(22)**

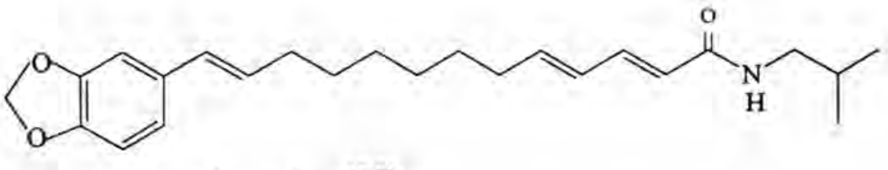
Fig.1.3 (cont.)



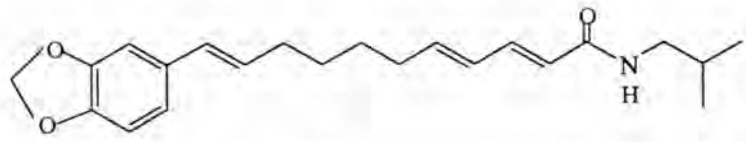
quassin **(23)**



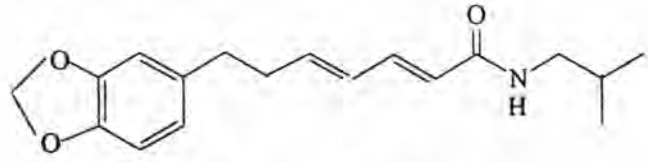
isobrucein - B **(24)**



guineensino **(25)**

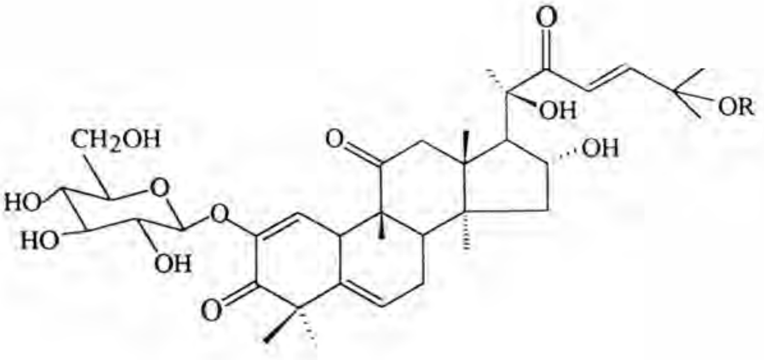


pipercide **(26)**



chingchengenamide A **(27)**

Fig. 1.4 Quassinoid class antifeedants



2-*O*-β-D-glucopyranosyl cucurbitacin E **(28)** R = $COCH_3$

2-*O*-β-D-glucopyranosyl cucurbitacin I **(29)** R = H

Fig. 1.5 Cucurbitacin class antifeedants

The greater wax moth, *Galleria mellonella* Linn., is a pest of stored honey combs and commercial beewax rather than in nature. It can also damage combs in active, colonies of the honey bee, *Apis mellifera* L. Female adults lay eggs on or near the bee wax. Developing larvae feed and burrow through the comb. The moth larvae often destroy unprotected combs in storage facilities or in colonies that become weakened or die. The feeding damage either makes the comb unusable by bees or requires the bees to reconstruct the comb. Losses due to this pest have been estimated to about $ 4 million per year in United States, as a combination of labor costs wax replacement, and lost revenues due to the carbohydrate consumption requirement of bees rebuilding combs (Williams, 1976).

Moreover, in tropical climate, the other warm areas, larvae of the greater wax moth cause extensive loss because they may develop continuously throughout the year (Smith 1960, Whitcomb, 1942).

CLASSIFICATION

Kingdom Animalia
Phylum Arthropoda
Class Insecta
Order Lepidoptera
Family Pyralidae
Genus Galleria
Species *Galleria mellonella* Linn.

(Borror *et al.*,1976)

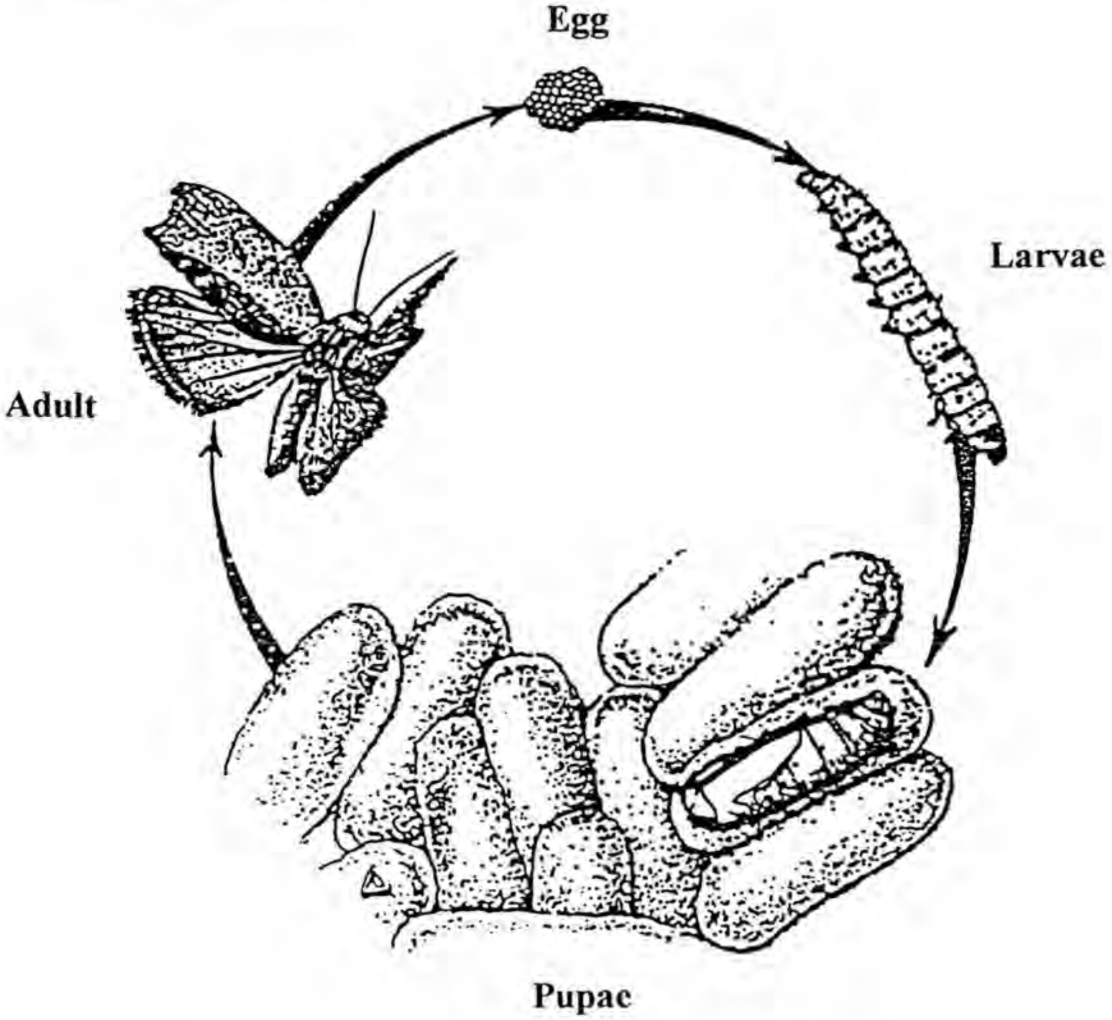


Fig. 1.6 Life cycle of *G. mellonella* Linn. (USDA, 1970)

Greater wax moth passes through four distinct stages: the egg or ovum (embryo stage); the caterpillar or larva (growing stage); the chrysalis or pupa (transition stage); and finally the adult or imago (reproduction stage). This four-stage development in insect is known as complete metamorphosis (Signh, 1962).

Egg: Sperical, smooth, creamy white, 0.4 to 0.5 mm, in size and laid in clusters hidden in cracks and crevices in the hive and of the comb and only occasionally on open surfaces if incubated at a temperature of 93 ° F, they will usually hatch in 3 to 4 days.

Larvae: Freshly hatched larva is white and 3 mm in length; full grown is dirty gray and up to 30 mm long; its brownish head is conspicuous. The larvae feed on honey within an hour of hatching and many burrow into pollen or cell walls. They live in the silken tunnels spun by themselves. The larval period lasts from about 18 days to 3 months, during which a maximum body length of about 2.5 cm (1 inch) is reached. The final size of larvae is largely dependent on temperature and the type and amount of food available.

Pupa: The young pupa is brownish white and the old dark brown; 14 to 16 mm long; pupal case usually white, often covered by scattered pieces of faces and frass, up to 28 mm long. The cocoons usually form a mass strongly webbed together in rows or tiers, sometimes actually carved in the wooden parts of the hive.

Adult: Brownish gray; 10 to 18 mm in length; wing expense 15 to 40 mm; the female larger than the male. A wax moth female produces less 300 eggs during her life span of 3 to 30 days, but a few may lay as many as 2000 eggs. The colour and size of adults varies a great deal in accordance with the food eaten during the larval period; dark brown combs containing pollen give rise to darker and bigger adults. The outer margin of the front wings of the males has a semi-lunar notch, whereas that of the female is smooth.

Methods of controlling *Galleria mellonella*

Several methods of wax moth control that are currently used as well as several methods that have been proposed for use could be summarized as follows :

1. Chemical control methods include various fumigants, such as *p*-dichlorobenzene, methylbromide and phosphine; fumigation with carbon dioxide or ethylene oxide. (Burges, 1978 and Cantwell, 1980).

- *p*-Dichlorobenzene: it was used to kill larval and adult stages of the wax moth but its does not kill the egg stage of the insect; hence it cannot be relied on the rid the area completely of the pest.

2. Physical control

- Temperature : it is possible to kill all life stages of the wax moth by exposing them to 0-5 °F for as little as 2 hours. It is also possible to kill this insect with high temperature. Exposure to 115-120 °F will produce 100% mortality in as little as one and a half hours.

3. Biological control for example, a braconid wasp, *Apanteles galleriae* Wilkinson, various species of predaceous ants, especially the red imported fire ant, *Solenopsis invicta* Buren, and an entomorphilous bacterium, *Bacillus thuringiensis* Berliner (Gershenson 1957 and Lavie *et al.* 1965), *B. thuringiensis* gave promising results against the wax moth on stored comb, both in the laboratory and on stored equipment, by using a strain of the bacterium that had until then been untested against the wax moth in the field, such as Certan (Sandoz, Crop Protection, San Diego, Calif.) is a liquid concentrate formulation of *B. thuringiensis* serotype 7 (Cantwell and Shieh 1981). Both spores and crystal endotoxin of serotype 7 are active components against greater wax moth (Jarrett and Burges 1982). *Fusarium sambucinum* Fuckel showed antifeedant activity towards larvae of *G. mellonella*. The activity appeared mostly due to the concentration of trichothecenes present in the fungal extracts (Mule *et al.* 1992).

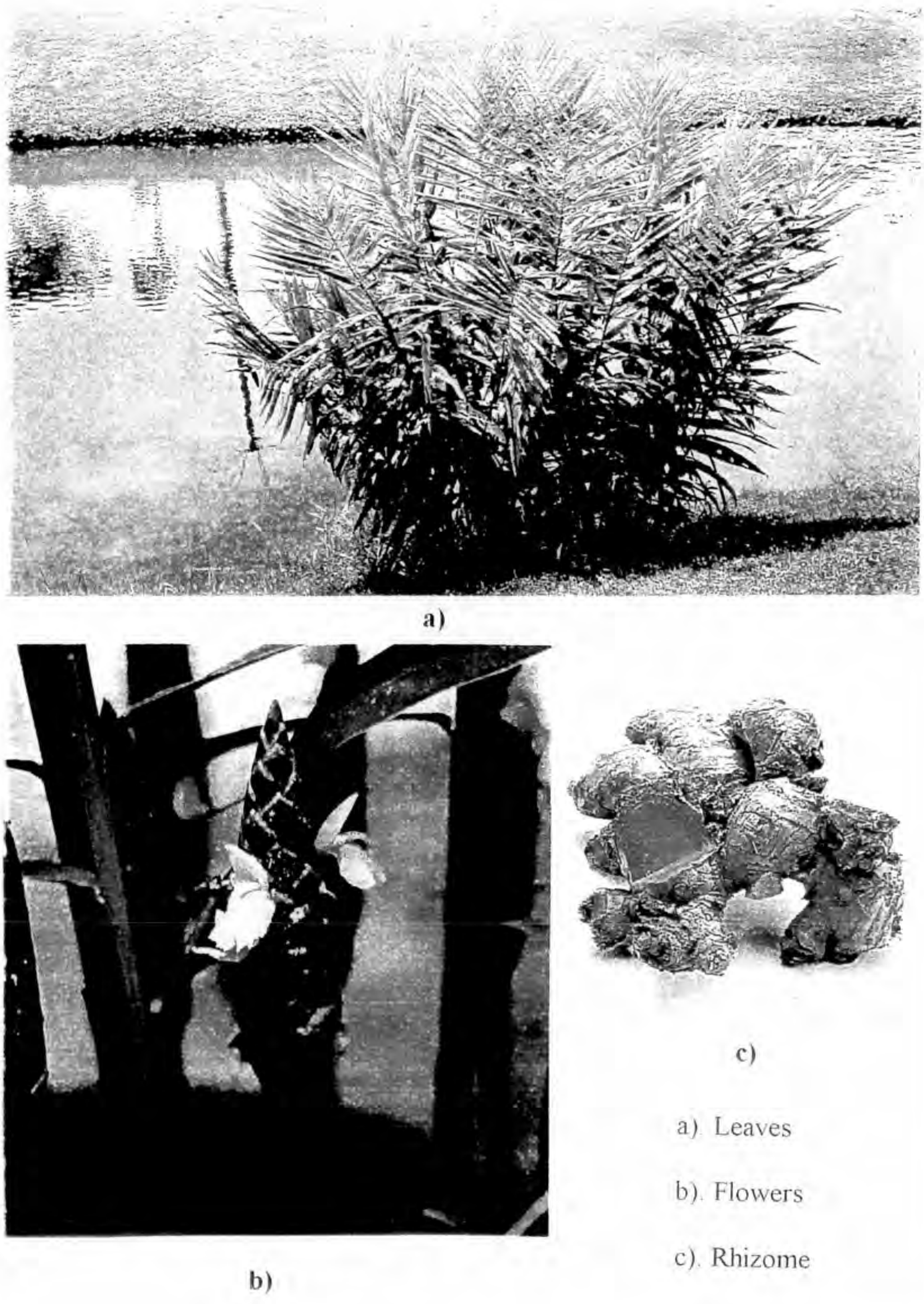
4. Control of the greater wax moth by beekeeper is simply a matter of proper colony management favoring strong populations (maintaining young, productive queens and adequate food stores, controlling disease, *etc.*) (Anderson, 1969).

1.2 Literature Search on the Chemical Constituents of *Zingiber cassumunar* Roxb.

Z. cassumunar Roxb. belongs to family Zingiberaceae (Ginger Family). The ginger family, known as the source of "ginger root" from plants of the genus Zingiber, and of cardamon seed from Elettaria, contains 47 genera of 1,400 tropical and subtropical species. They are primarily perennial herbs with horizontal or tuberous rhizomes (Langenheim and Thimann, 1982). Furthermore, members of the Zingiberaceae are famous for their uses as spices or as medicinal herbs. Well-known example include the rhizomes of *Z. officinale* (ginger) or *Curcuma longa* (syn. *C. domestica*) (turmeric) for treatment of scabalous constipation, for promoting longevity, as a digestive stimulant, as a carminative, and for relief of nausea and vomiting. *Z. zerumbet* Smith. (Ka-thue) treatment of sprain and bodily discomfort, abdominal discomfort and enhancing appetite. It has also been found to be efficient as an antibacterial, an anti-inflammatory, antiemetic and mutagenic activity. In addition, *Z. rubens* Roxb. was used as an ingredient in a traditional "YaaHom" (fragrant medicine) used in Sukothai and Pitsanulok Provinces.

In Thailand, Zingiber genus has many species but some of the most common species are *Zingiber officinale* Rosc. (Khing), *Zingiber cassumunar* Roxb. (Phlai),*Zingiber spectabile* Griff. (Phlai-muaug), *Zingiber offensii* Val. (Phlai-dam), *Zingiber rubens* Roxb. (Mayee) and *Zingiber zerubet* Smith (Ka-thue).

The common name of *Z. cassumunar* Roxb. in Thailand is "Phlai" or "Waan fai" (central region), "Puu loi" or "Puu loei" (northern region) and "Min-sa-laang" (Shan-Mae Hong Son) (Smitinand, 1980). *Z. cassumunar* Roxb. is a rhizomatous herbs, 0.7-1.5 m high, characteristic odor. Leaves are simple, alternate, lanceolate-oblong, 3.5-5.5 cm wide, 18-35 cm long. Inflorescence in the terminal spike, from apex of the rhizome; flower white or yellowish white; fruit globose capsule (Saralamp and *et al.*, 1996) (Fig. 1.7) .



a)

b)

c)

a). Leaves

b). Flowers

c). Rhizome

Fig. 1.7 *Zingiber cassumunar* Roxb.

1.3 Utility of *Z. cassumunar* Roxb.

The rhizomes of *Z. cassumunar* Roxb. are used for carminative, emmenagogue, mild laxative, antidysenteric, stringent; externally used as anti-inflammatory for a sprins, muscular pain and wound-healing. Volatile oil from rhizome has anti-inflammatory effect. In a mixture, it could be used as mosquito repellant and 4-(4-hydroxy-1-butenyl) veratrole, a constituent in rhizome shows bronchodilatic activity. Powdered rhizome exhibits bronchodilating effect in both chronic and acute asthma in children. In addition some compounds of rhizome extraction can be used as an antioxidant such as cassumunin A-C and zerumbone for fungitoxic action against *Rhizoctonia solani* (the damping off pathogen).

Among those attractive biological activities, the crude extracts from rhizomes of 19 different species of the Zingiberace, *Z. cassumunar* shows high insecticidal activity. Two phenylbutanoids **(50,51)** were isolated from rhizomes of *Z. cassumunar* and were found to possess the activity against neonate larvae of pest insect, *Spodoptera littoralis*.

1.4 Chemical Constituents Studies on *Zingiber cassumunar* Roxb.

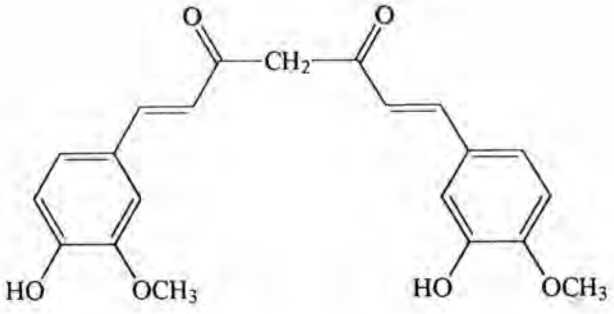
Literature surveys of chemical constituents of the plants belonging to Zingiber genus revealed that there have been a variety of organic substrates isolated (Table 1.2). The structures of some isolated compounds are shown in Fig. 1.8.

Table 1.2 Chemical constituents of *Z. cassumunar* Roxb.

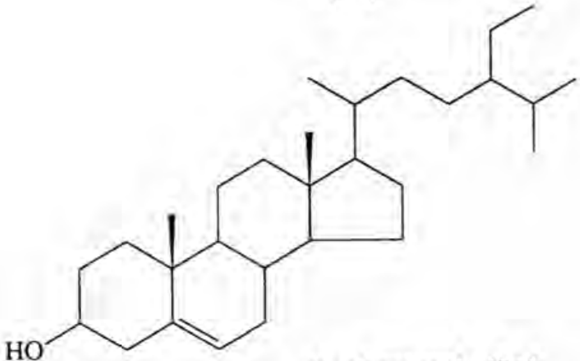
Plant parts	Crude extract	Isolated compounds	References
rhizome	chloroform	curcumin **(30)**	Thanomwang and Kunitoshi (1973)
		β-sitosterol **(31)**	
	hexane	β-sitosterol **(31)**	Tuntiwachwuttikul *et al.* (1981)
		4-(3′,4′ - dimethoxyphenyl)but-1,3-diene **(32)**	
		4-(3′,4′-dimethoxyphenyl)but-3-ene **(33)**	
		4-(2′,4′,5′-trimethoxyphenyl)but-1,3-diene **(34)**	
		4-(2′,4′,5′ -trimethoxyphenyl)but-3-ene **(35)**	
		(*E*)-4-(3′,4′-dimethoxyphenyl)but-3-en-1-yl palmitate **(36)**	
		3,4-dimethoxybenzaldehyde **(37)**	
		2,4,5-trimethoxybenzaldehyde **(38)**	
		(*E*)-4-(3,4-dimethoxyphenyl)-but-3-en-1-ol	Kanjanapothi (1986)
		(*E*)-1-(3,4-dimethoxyphenyl)-but-1-ene	Ozaki *et al.* (1991)
		(*E*)-1-(3,4-dimethoxyphenyl)-1,3-butadiene	
		zerumbone	

Table 1.2 (cont.)

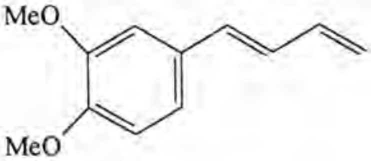
Plant parts	Crude extract	Isolated compounds	References
	toluene	3-aryl-4-styrylcyclohexane **(39)** cassumunene	Dinter (1980)
	acetone	(±)-*trans*-3-(2,4,5-trimethoxyphenyl)-4-[(*E*)-3,4-dimethoxystyryl]-cyclohexane **(40)**	Jitoe *et al.* (1993)
		cis-1,2-*bis* [(*E*)-3,4-dimethoxystyryl] butane **(41)**	
		cassumunin A,B,C **(42,43,44)**	Masuda *et al.*(1993)
	ethyl acetate	(*E*)-4-(3,4-dimethoxyphenyl)-but-3-en-1-ol **(45)**	Masuda and Jitoe (1995)
		(*E*)-4-(3,4-dimethoxyphenyl)-but-3-en-1-yl acetate **(46)**	
		(*E*)-3-hydroxy-1-(3,4- dimethoxyphenyl)but-1-ene **(47)**	
		(*E*)-4-(4-hydroxy-3-methoxyphenyl)-but-3-en-1-yl acetate **(48)**	
		(*E*)-4-(4-hydroxy-3-methoxyphenyl) but-2-en-1-ol **(49)**	
		1,2-dimethoxy-4-(3-butanol)benzene **(50)**	Nugroho *et al.* (1996)
		1,2-dimethoxy-4-(4-acetylbutene) benzene **(51)**	



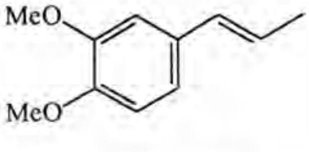
curcumin **(30)**



β- sitosterol **(31)**

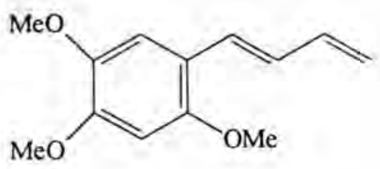


4-(3',4'- dimethoxyphenyl)but-1,3-diene **(32)**

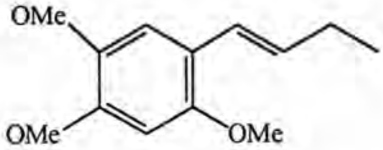


4-(3′,4′-dimethoxyphenyl)but-3-ene **(33)**

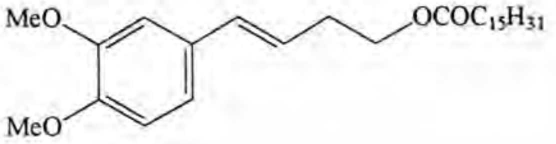
Fig. 1.8 Some compounds isolated from rhizomes of *Zingiber cassumunar* Roxb.



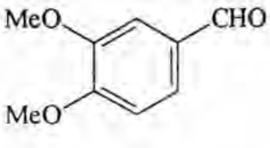
4-(2′,4′,5′-trimethoxyphenyl)but-1,3-diene **(34)**



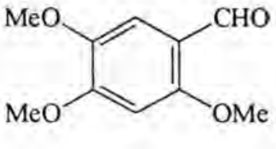
4-(2′,4′,5′-trimethoxyphenyl)but-3-ene **(35)**



(*E*)-4-(3′,4′-dimethoxyphenyl)but-3-en-1-yl palmitate **(36)**

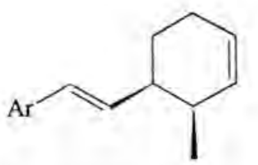


3,4-dimethoxybenzaldehyde **(37)**



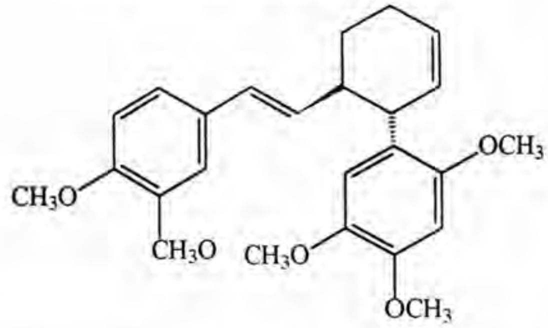
2,4,5-trimethoxybenzaldehyde **(38)**

Fig. 1.8 (cont.)

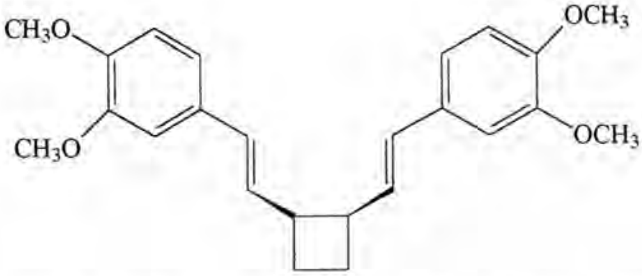


Ar = 2,4,5-trimethoxybenzaldehyde

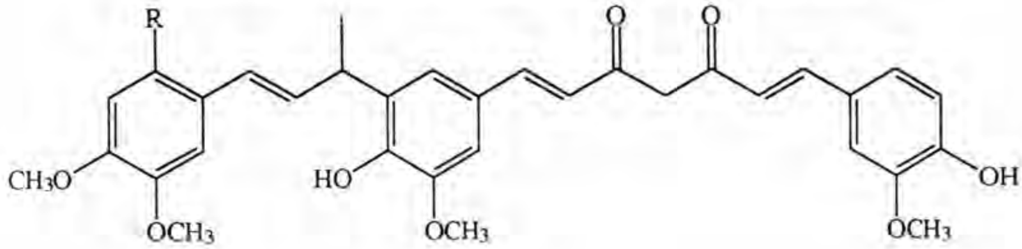
3-aryl-4-styrylcyclohexane **(39)**



(±)-*trans*-3-(2,4,5-trimethoxyphenyl)-4-[(*E*)-3,4-dimethoxystyryl]-cyclohexane **(40)**



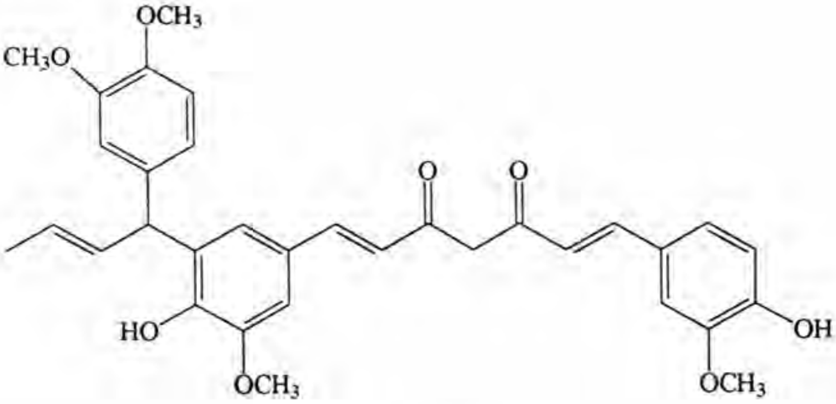
cis-1,2-*bis* [(*E*)-3,4-dimethoxystyryl]cyclobutane **(41)**



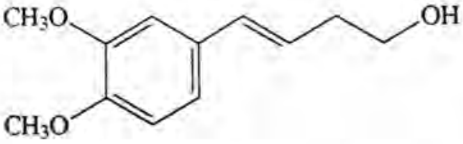
cassumunin A and B

R = H **(42)** ; R = OCH_3 **(43)**

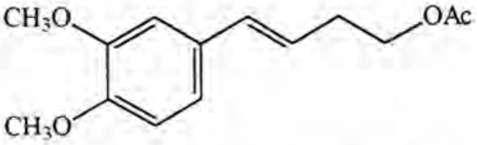
Fig. 1.8 (cont.)



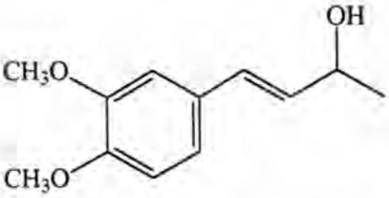
cassumunin C **(44)**



(*E*)-4-(3,4-dimethoxyphenyl)- but-3-en-1-ol**(45)**

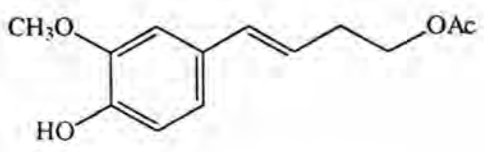


(*E*)-4-(3,4-dimethoxyphenyl)-but-3-en-1-yl acetate **(46)**

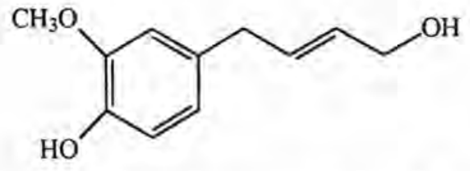


(*E*)-3-hydroxy-1-(3,4-dimethoxyphenyl)-but-1-ene **(47)**

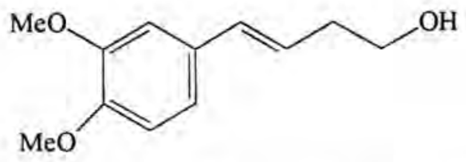
Fig. 1.8 (cont.)



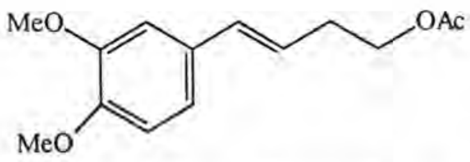
(*E*)-4-(4-hydroxy-3-methoxyphenyl)-but-3-en-1-yl acetate **(48)**



(*E*)-4-(4-hydroxy-3-methoxyphenyl)-but-2-en-1-ol **(49)**



1,2-dimethoxy-4-(3-butenol)benzene **(50)**



1,2-dimethoxy-4-(3-acetylbutene)benzene **(51)**

Fig. 1.8 (cont.)

1.5 Goal of This Research

The goal of this research could be summarized as follows :

1. Preliminary screening test for antifeedant activity against *Galleria mellonella* Linn. of 17 species from Thai plants.

2. To extract and fractionation the organic compounds from the plants

3. To elucidate the structural formulae of the isolated substances from the most attractive ethanolic crude extract.

4. To search for antifeedant compounds against 3 rd instar larvae of the greater wax moth, *G. mellonella*.