10 Plant Natural Products as a Source for Developing Environmentally Acceptable Insecticides

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1 Introduction

Terrestrial plants produce a bewildering array of natural products—terpenoids, phenolics, alkaloids—likely exceeding 100,000 novel chemical structures. Many of these are thought to serve an ecological function for the plants producing them, serving to defend the plants from herbivores and pathogens. Such defensive chemistry is thought to be extremely widespread among the plant kingdom. This is the foundation upon which many scientists have viewed higher plants as a valuable resource that could be exploited for the discovery of new insecticides or for novel structures that could serve as lead compounds in insecticide development.

This rationale is based partly on the assumption that novel structures produced by plants may have novel modes-of-action, and that some or many of these may exhibit selectivity favoring mammals, given that insects are thought to be major agents of natural selection for plants and therefore defensive chemistry is more likely targeted towards insects than towards vertebrate herbivores. Several volumes have been published around the concept of natural insecticides from plants (Arnason et al. 1989; Hedin et al. 1997; Koul and Dhaliwal 2001; Regnault-Roger et al. 2005).

In reality, natural insecticides from plants have proven to be the exception rather than the rule, based on the conventional use of the term "insecticide"—a substance that kills insects. Although hundreds of compounds isolated from plants have demonstrated bioactivity to one or more insects based on bioassays in the laboratory, feeding deterrence or larval growth inhibition is far more commonly seen than death of the insects tested. This may partially explain why only a handful of botanical insecticides have been commercialized in industrial countries—in seeking bioactivity comparable to synthetic insecticides (i.e., acute toxicity to insects), very few plant natural products with satisfactory insecticidal activity have been identified from the thousands of plant extracts screened. In short, plant defensive chemistry has probably evolved to discourage insect (and mammalian) herbivory, rather than to kill the herbivores outright.

In this chapter we consider several plant natural products with insecticidal activity, review their structure–activity relations, and discuss efforts aimed at enhancing their bioactivity (efficacy and/or spectrum of action).

Insecticides Design Using Advanced Technologies (ed. by Isaac Ishaaya, Ralf Nauen and A. Rami Horowitz)

2 From Pyrethrum to Synthetic Pyrethroids

The development of the synthetic pyrethroids, arguably one of the most commercially successful classes of conventional insecticides, is often cited as an example of how a plant natural product can serve as a lead in the development of crop protection agents. However, it must be remembered that the first commercially significant pyrethroids—those used for crop protection in the field (e.g., permethrin and fenvalerate)—were not discovered until 1973. This was almost 25 years after the synthesis of the first synthetic pyrethroid, allethrin (in 1948), and nearly 60 years after the structural elucidation of the natural pyrethrins. What made the synthetic pyrethroids useful for agriculture was their reduced photolability in the presence of sunlight through halide substitution of the parent chrysanthemic acid and replacement of the butenolide ring with a more stable 3-phenoxyphenyl ring system (Fig. 1). As such, the modern pyrethroids bear little resemblance to their natural product progenitor (pyrethrin I), and even the mode-of-action of the later pyrethroid insecticides differs from the original natural product (Perry et al. 1998).

3 Azadirachtin and Related Limonoids from the Meliaceae

No insecticide of plant origin has been subjected to as much scrutiny in the past 20 years as that of neem, derived from the seeds of the Indian neem tree *Azadirachta indica* (Schmutterer 2002; Koul and Wahab 2004). Seeds of *A. indica* produce a suite of closely related limonoid triterpenes, the most important of which is azadirachtin (**1**, Fig. 2). In purity, this substance remains the most potent insect antifeedant discovered to date, at least in bioassays using the desert locust *Schistocerca gregaria* and some species of noctuid caterpillars. Antifeedant effects of azadirachtin (and other antifeedants of plant origin) vary widely in their potency to different insect species; some are behaviorally quite indifferent to it. Of greater value for insect control are the exceptional growth regulatory actions of this compound on most types of insect. Through interference with the insect neuroendocrine system, azadirachtin disrupts moulting and metamorphosis, blocks reproduction, and can cause anorexia in some species.

Azadirachtin was first isolated in 1968 (Butterworth and Morgan 1968), but its structure was not conclusively determined until several years later (Kraus et al. 1987). Rembold (1989) conducted one of the first structure–activity studies of the natural analogues of azadirachtin in neem seeds. Azadirachtin, relatively the most abundant among approximately one dozen closely related analogues, is also the most biologically active in most insect-based bioassays. Two very minor constituents (azadirachtins H and I) were found to be about twice as active as azadirachtin based on the *Epilachna* molting bioassay. Rembold (1989) also defined minimal structure requirements of the azadirachtin molecule for insect growth regulatory activity.

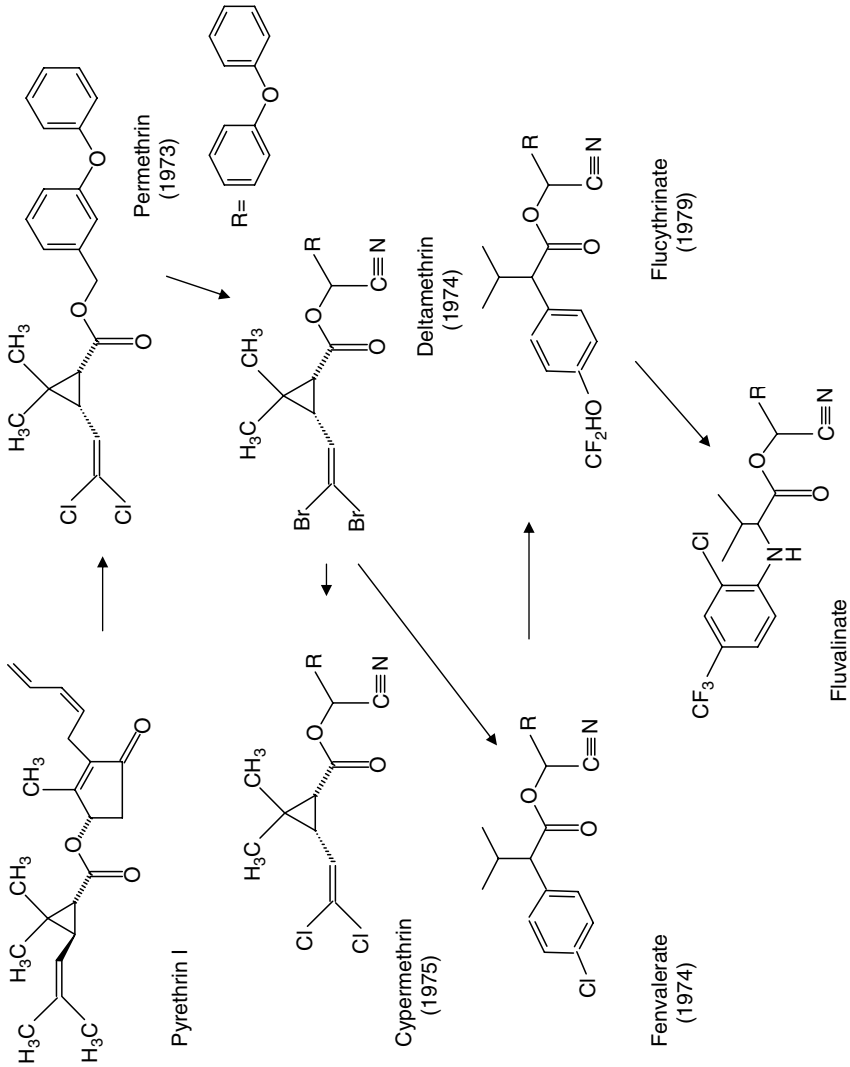

Fig. 1. "Evolution" of synthetic pyrethroids derived from the plant natural product pyrethrin I

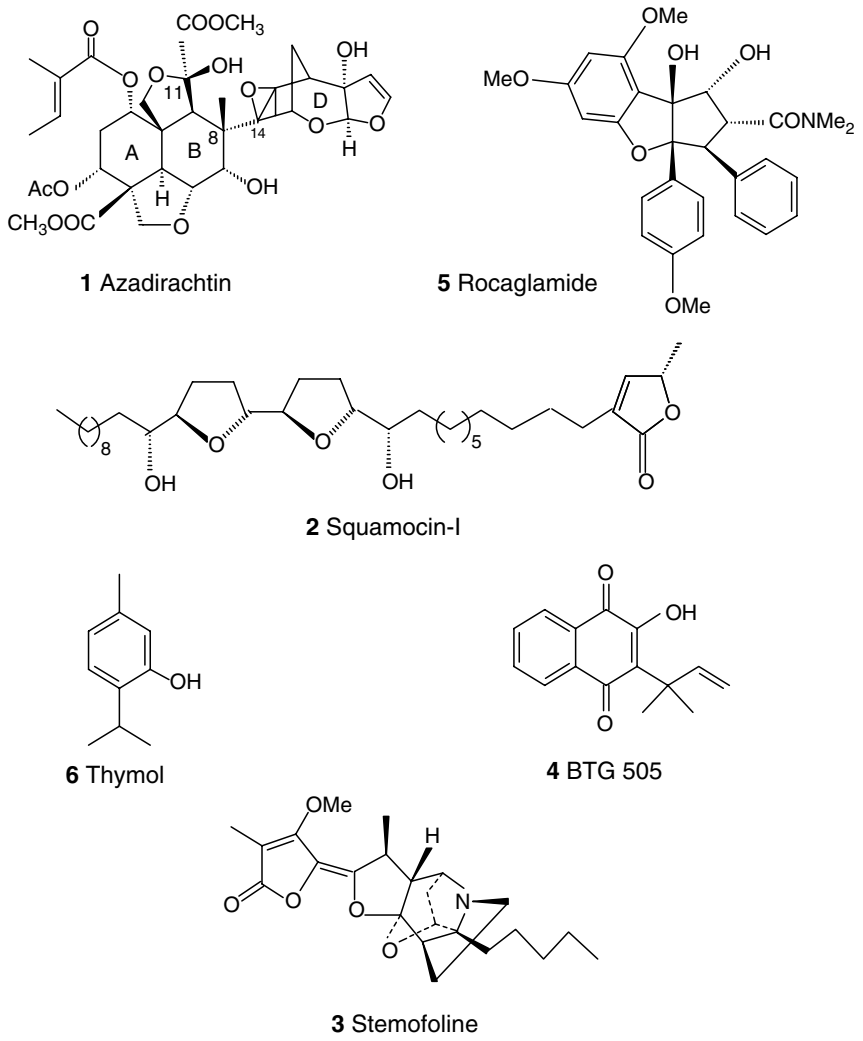

Fig. 2. Some insecticidal plant natural products discussed in this chapter

These observations were extended by Blaney and Simmonds, in collaboration with Ley (Blaney et al. 1990; Simmonds et al. 1995; Ley 1994). Almost 60 analogues, both natural and synthetic derivatives, have been investigated with respect to antifeedant and insecticidal actions on locusts and noctuid larvae. One important conclusion from this body of work is that almost all analogues are less potent than the parent compound azadirachtin, largely confirming Rembold's investigations. Another is that specific binding of azadirachtin analogues to membrane proteins, based on studies with insect cell cultures in vitro, correlates well with observations on insect behavior and

physiology in intact insects (Mordue [Luntz] et al. 1998). Of particular interest have been efforts by Ley and colleagues to synthesize azadirachtin de novo, a formidable undertaking given the large number of chiral centers and overall complexity of the molecule. Their strategy has been to synthesize the substituted decalin ("western") fragment and to synthesize the dihydrofuranacetal ("eastern") fragment, with the intent to link the two fragments through the crucial C8-C14 bond.

Antifeedant bioassay of the separate fragments in the cotton leafworm *Spodoptera littoralis* indicated that the decalin fragment possessed some bioactivity by itself (at 10^{-6} M), and that certain combinations of decalin and furanacetal fragments were synergistic (also at 10^{-6} M). However, in the same insect, azadirachtin is active as an antifeedant at 10^{-15} M; one could argue that compounds active only at concentrations nine orders of magnitude greater are hardly active at all.

To date, the total synthesis of azadirachtin has not been accomplished on any meaningful scale. Alternatives to synthesis include the production of this complex molecule through plant tissue culture, but to this point, extraction from neem seeds is the only commercially viable route to production of neem insecticides. Moreover, the use of neem or azadirachtin from natural sources has yet to achieve its' promise in the marketplace, even 15 years after its commercialization in the United States (Isman 2004). So while azadirachtin has proven to be a fascinating molecule for the study of insect feeding behavior and endocrine physiology, it has not been a fruitful lead for the production of synthetic insecticides to this point.

4 Acetogenins from the Annonaceae

Derivatives from species of the custard apple family have had a number of traditional uses for pest control and as vermifuges (reviewed in McLaughlin et al. 1997). These actions were erroneously attributed to a series of benzylisoquinoline alkaloids isolated from annonaceous plant extracts. Bioassay-driven fractionation employing a simple brine shrimp assay (*Artemia salina*) led to the isolation and elucidation of C-32 or C-34 long-chain fatty acids combined with a 2-propanol unit at C-2 to form an α-lactone, as the active principles (Mikolajczak et al. 1988, 1989). To date, more than 400 structures of this type have been isolated from about 40 species of the Annonaceae, particularly from the bark, fruit, and seeds. Species that have been more intensively examined include the temperate American paw paw (*Asimina triloba*) and the tropical sour sop (*Annona muricata*) and sweet sop (*Annona squamosa*). Each of these species contain complex mixtures of acetogenins comprising at least 30 compounds (e.g., squamocin, **2**, Fig. 2).

McLauglin and colleagues investigated structure-activity relations among 44 acetogenins isolated from *Asimina triloba, A. longifolia, Annona muricata,*

A. bullata, Goniothalamus giganeus and *Rollinia mucosa* (He et al. 1997), using yellow fever mosquito larvae (*Aedes aegypti*). Rotenone, a commercial botanical insecticide from *Derris elliptica* and related tropical legumes, was included as a positive control. The acetogenins, as inhibitors of mitochondrial NADH:ubiquinone oxidoreductase, have the same mechanism of action as rotenone, making the comparison particularly appropriate (Londershausen et al. 1991). Among the 44 acetogenins, two were significantly more toxic to mosquito larvae than rotenone, while a further 28 were statistically equitoxic to rotenone (LC_{50} = 1.2 ppm) (He et al. 1997). Compounds with adjacent bis-tetrahydrofuran rings and three hydroxyl groups were the most active; those with a mono-tetrahydrofuran ring and a single flanking hydroxyl group were the least active.

Although three patents based on the use of these substances for insect control were issued more than 15 years ago, no substantial commercialization has ensued. As mitochondrial poisons toxic to a wide range of organisms, including mammals, the acetogenins are not considered attractive lead molecules for synthetic efforts. As for the azadirachtins, the main use of the acetogenins for insect control will probably continue to be based on crude or partially refined extracts obtained from plant sources (Leatemia and Isman 2004).

5 Alkaloids from Stemonaceae

The family Stemonaceae, the only source of stemona alkaloids (Pilli and Ferreira de Oliviera 2000) is a small monocotyledonous family with three genera comprising about 30 species. *Stemona* is the largest genus with about 25 species occurring as subshrubs or twining herbs, mostly with perennial tuberous roots (Brem et al. 2002, 2004).

The stemona alkaloids include more than 70 different derivatives that have been classified into eight different groups by Ye et al. (1994) according to the sites of connection between the basic ring and side chain. Structurally, stemona alkaloids are characterized by a pyrollo(1,2-α)azepine (5,7 bicyclic AB-ring system) nucleus (Brem et al. 2002) common to all compounds in six of these groups or a pyrido(1,2-α)azepine nucleus (6,7-bicyclic AB-ring system) (Kaltenegger et al. 2003) in the more recently discovered stemoncurtisine group of stemona alkaloids.

Extracts from *Stemona* and *Croomia* have been used for centuries in Chinese and Japanese traditional medicines for the treatment of respiratory diseases and against enteric helminths and ectoparasites on humans and cattle (Xu et al. 1982; Sakata et al. 1978) and as insecticides (Xu, 2000; Brem et al. 2002; Kaltenegger et al. 2003).

Strong biological activity was exhibited by the crude extracts from the roots of *Stemona cochinchinensis* and *S. curtisii* against *Spodoptera littoralis* (Kaltenegger et al. 2003) and may be attributed to the presence of the alkaloid

stemofoline (**3**, Fig. 2). Moreover, crude extracts of *Stemona* species were almost as active as the isolated compounds. Crude extracts from *S. collinsae* displayed the highest insecticidal activity against *S. littorallis*. These were more active than extracts from two very active *Aglaia* species (Meliaceae) or a standard pyrethrum extract. The high activity of *S. collinsae* was attributed to the predominance of didehydrostemofoline accompanied by smaller amounts of stemofoline. In contact toxicity bioassays, the insect-toxic potencies of stemofoline and didehydrostemofoline exceeded even those of the pyrethrum extract. Stemofoline's mode of action is agonism of the insect nicotinic acetylcholine receptor (Godfrey et al. 2002).

Kaltenegger et al. (2003) investigated structure–activity relations of 13 stemona alkaloids based on insecticidal activity against neonates of *S. littoralis*. Modification or absence of the unsaturated lactonic 4-methoxy-3-methyl-2-furanone unit from stemocochinin and parvistemonine led to a strong decrease in toxicity. With regard to the five novel pyridoazepine derivatives, oxystemokerrin with an oxygen bridge between the C-1 and C-8 of the pyridoazepine nucleus displayed the strongest activity. Loss of the open butyl side chain resulted in the reduction of toxicity in pyridostemin. N-oxidation in oxystemokerrin-N-oxide or insertion of a double bond in the azepine ring of stemokerrin decreased activity. However, insertion of a double bond between C-7 and C-8 in dehydroprotostemofoline (pyrroloazepine derivative) increased toxicity. All these examples suggest that modification of the chemical structure of the parent compound leads to decreased biological activity of the analogues.

6 Napthoquinones from the Scrophulariaceae

A 5-year collaborative project led by researchers at IACR-Rothamsted (UK) resulted in the isolation of two active principles from the Chilean plant *Calceolaria andina* (Scrophulariaceae), related to the familiar garden "slipper" plants. These compounds (BTG 504 and BTG 505), identified as napthoquinones, are effective against a range of commercially important pests including the tobacco whitefly, *Bemisia tabaci,* aphids and the two-spotted spider mite, *Tetranychus urticae* (Khambay et al. 1995; 1999). They offer opportunities both as lead structures for analogue synthesis (Khambay et al. 1997a) and as new botanical pesticides (Khambay et al. 1997b) exhibiting low mammalian toxicity unlike other naphthoquinones. The use of the compounds as pesticides has been patented (Khambay et al. 1999) by BTG International Ltd.

Khambay et al. (2003) compared the insecticidal and fungicidal properties of dunnione (a known naphthoquinone) with BTG 505 (**4**, Fig. 2). Although, dunnione showed practically no activity against the house fly *Musca domestica,* the whitefly *B. tabaci*, the beetle *Phaedon cochleariae*, or the spider mite

T. urticae unlike BTG 504 and BTG 505, dunnione has an unusually broad spectrum of antifungal activity. The mode of action of dunnione is primarily through initiation of redox cycling, whereas, BTG 505 acts by inhibiting mitochondrial complex III (Khambay et al. 2003).

The biological activity of 2-hydoxy-3-subsituted-1,4-naphthoquinones was first reported by Fieser et al. (1948) showing that lapachol and hydrolapachol were active against *Plasmodium lophurae*, a malarial parasite of ducks. The recently developed antimalarial drug, atovaquone is a synthetic analogue of lapachol (Olliao and Trigg 1995). Antifungal activity of lapachol obtained from the wood extract of *Tabebuia serratifolia* (Bignoniaceae) was reported by Velasquez et al. (2004). Lapachol obtained from an ethanolic bark extract of *T. serratifolia* was more active (LC_{50} = 20.8 ppm), than the amine derivatives (LC_{50} values ranged from 242.6–899.4 ppm) against the larvae of *Aedes aegypti,* showing the importance of the hydroxyl group at the C-2 position for bioactivity (Oliviera et al. 2002). An outstanding property of these compounds is that they are effective against a range of resistant insect strains including the notorious B-biotype of the tobacco whitefly, *Bemisia tabaci*, which is devastating crops worldwide.

7 Rocaglamides from *Aglaia* (Meliaceae)

The genus *Aglaia* consisting of some 130 species widely distributed in the Indo-Malysian region (Nugroho et al. 1999) has attracted considerable attention in the past decade as a possible source of unique natural products. Phytochemical investigations of *Aglaia* have revealed the presence of a variety of compounds including rocaglamides (Ishibashi et al. 1993; Proksch et al. 2001), aglains (Bacher et al. 1999), bisamides (Brader et al. 1998), triterpenes (Weber et al. 2000) and lignans (Wang et al. 2002) with interesting biological activities.

There are more than 50 naturally occurring rocaglamide derivatives isolated to date (e.g., rocaglamide, **5**, Fig. 2) (Proksch et al. 2001). Rocaglamide derivatives are unusual aromatic compounds featuring a cyclopentatetrahydrobenzofuran skeleton and are strictly confined to members of *Aglaia*. Recently, several novel rocaglamide derivatives, isolated from different *Aglaia* species have been shown to have strong insecticidal activity (in some cases even comparable to azadirachtin), mostly against neonate larvae of *Spodoptera littoralis*, *Ostrinia* species and the gram pod borer, *Helicoverpa armigera* (Brader et al. 1998; Nugroho et al. 1997a, 1997b, 1999; Gussregan et al. 1999; Koul et al. 2004). The insecticidal mode-of-action as well as the potential anit-cancer activity of rocaglamides results from inhibition of protein synthesis, explaining the long time-to-death in treated insects (Satasook et al. 1993).

The insecticidal activity of rocaglamides can be attributed to the presence of the furan ring system since the closely related aglains, possessing a pyran ring, are devoid of insecticidal activity (Nugroho et al. 1999). The nature of

the substituents at C-1, C-2, C-3 and C-8b have also been suggested to be responsible for the bioactivity of the respective derivatives (Nugroho et al. 1997a, 1999; Schneider et al. 2000). Acylation of the OH group (with formic or acetic acid) at C-1 caused a reduction of insecticidal activity in neonate larvae of *S. littoralis* compared with other rocaglamide derivatives with a hydroxyl substituent isolated from the twigs of *A. duppereana* (Nugroho et al. 1997a). The reduction of insecticidal activity in the acetylated derivative indicates the first structure–activity relationship in this group of natural insecticides. There is a decline in insecticidal activity for rocaglamide derivatives featuring an unsubstituted C-2 in contrast with analogues possessing an amide or carboxylic substituent at this position. A similar trend has been noted in other rocaglamide derivatives isolated from *A. odorata* (Nugroho et al. 1999; Gussregan et al. 1997), and *A. elliptica* (Nugroho et al. 1997b). Substitution of a hydroxy group with the methoxy group at C-8b resulted in a complete loss of activity in compounds that were isolated from roots of *A. duperreana* (Chaidir et al. 1999) showing the importance of the OH group at C-8b. The strong bioactivity of rocaglamide derivatives against a number of insect pests suggests that they may serve as lead structures in the development of natural insecticides for plant protection.

8 Monoterpenoids from Plant Essential Oils

Plant essential oils, viz. steam distillates from certain aromatic and/or medicinal herbs or trees, have long been known to possess insecticidal or insect repellent properties, but only recently have some of the natural oils been commercialized for those uses. In broad terms, the monoterpenoids exhibit neurotoxicity to insects and mites on contact, but only at doses substantially greater than those obtained with natural pyrethrins or conventional insecticides. A more detailed examination of their efficacy and modes of action can be found elsewhere in this volume.

Coats and Rice explored structure–activity relations of naturally-occurring monoterpenoids based on topical, fumigant and ovicidal activities using the house fly (*Musca domestica*), red flour beetle (*Tribolium castaneum*) and southern corn rootworm (*Diabrotica undecimpunctata howardi*) as models (Rice and Coats 1994a). Activities varied with skeletal structure (cyclicity), amount of saturation and functional groups, but trends were somewhat inconsistent among the three species tested. In general, ketones tended to be more active than analogous aldehydes that were in turn more effective than the respective alcohols. Phenols were more active than saturated alcohols topically and as larvicides, whereas the saturated alcohols were more effective as fumigants.

This study was expanded to include a large number of synthetic derivatives obtained through acylation of alcohol substituents (Rice and Coats

1994b; Tsao et al. 1995). In most bioassays, pivalates were more active than acetates, although the latter were generally more effective as ovicides. Fluoroacetates were particularly effective as house fly fumigants. Derivitization resulted in 2–3 fold increases in toxicity in some bioassays, although among 55 compounds tested for topical toxicity to the house fly, the natural product, thymol (**6**, Fig. 2), was the most active; all seven thymol derivatives were significantly less toxic. Enhanced fumigant and ovicidal activities likely resulted from increased volatility and lipophilicity of the acylated derivatives.

Recent evidence for an octopaminergic mode-of-action for certain monoterpenoids (Enan 2001; Bischof and Enan 2004; Kostyukovsky et al. 2002), combined with their relative chemical simplicity may yet find these natural products useful as lead structures for the discovery of new neurotoxic insecticides with good mammalian selectivity.

9 Conclusions

In this chapter we have reviewed a number of plant natural products with insecticidal activities, some long known and others recently discovered. Only a handful of these have seen commercial use as botanical insecticides due to several limiting factors.

In the case of neem, high input cost (2.5–3 times more expensive than synthetic pyrethroids) helps explain why neem insecticides in the USA are currently positioned with an emphasis on high-value row crops (e.g., fresh market tomatoes) and greenhouse crops (Isman 2004). Although, neem-based insecticides enjoy a broad spectrum-of-action against pests (> 400 species), they lack efficacy against certain pests including some species of tephritid flies (apple maggot, cherry fruit fly). Owing to limited persistence on plants, multiple applications of neem may be necessary to achieve acceptable control against some important pests (e.g., codling moth on apple, bollworm on cotton), which may not be economically feasible (Isman 2004). Neem insecticides also lack contact action and work slowly, a situation often disheartening to growers used to synthetic pyrethroids or other contact toxins that kill pests in a matter of hours. Although the bioactivity of the dozen or so azadirachtin analogs isolated to date from neem is well documented, the contribution of the remaining limonoid constituents of neem kernels to overall efficacy of neem insecticides remains controversial (Isman 2002) and unfortunately has become a point of confusion and uncertainty for regulatory agencies charged with evaluating neem as a pesticide (Isman 2004). Regulatory approval has greatly limited the introduction of neem insecticides in Europe, particularly on food plants, owing to residue and environmental data requirements.

We have focused our review on structure–activity relations, both among naturally occurring analogues, and among synthetic derivatives, with a view

to the potential of these natural substances as leads for the development of synthetic insecticides, either with novel modes-of-action or with reduced environmental and human health impacts through other means.

In our opinion, the only successful example of this approach has been the development of the synthetic pyrethroids, an endeavor that took over 25 years to accomplish. A decade of research on total synthesis of azadirachtin failed to bear fruit; the chemical complexity of this molecule and the numerous structural requirements for insect-growth regulatory activity pose a formidable challenge to the synthetic chemist. Rocaglamide poses a similar challenge. However, less complex plant natural products with sufficient insecticidal action (naphthoquinones, monoterpenoids) may yet prove useful for the development of insecticides with novel modes-of-action.

Perhaps the strongest conclusion from our review is the optimization of plant chemical defenses on an evolutionary timescale—insecticidal active principles from plants are not easily improved upon! Furthermore, the insecticidal activity found in plants is often enhanced by the presence of suites of closely related compounds, acting synergistically or at the least diffusing selection by insect herbivores and thus forestalling the development of resistance in those insect populations.

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