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REVIEW

Aaron Dossey

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Cover
See Aaron T. Dossey, pp. 1737–1757.
Insects such as these, and their chemical defense mechanisms, are a huge potential source of new natural products for medicinal and other valuable applications. Pictured here are (clockwise from top left): *Anisomorpha buprestoides* (Archbold Biological Research Station, Florida); *Pseudophasma annulipes* (Madre de Dios, Peru); a larva of *Papilio polyxenes* (Shady Oak Butterfly Farm, Florida); *Taeniopoda eques* (Big Bend National Park, Texas).
Photographs by Dr. Aaron T. Dossey, *Nat. Prod. Rep.*, 2010, **27**, 1737.

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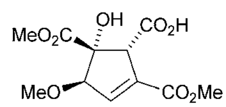

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REVIEWS

Insects and their chemical weaponry: New potential for drug discovery

Aaron T. Dossey*

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Insects make up the largest and most diverse group of organisms on earth, with nearly 1 million species described and millions more estimated to remain undiscovered. Like all other organisms, insects and related arthropods mainly utilize chemistry to adapt to these environments in a wide variety of ways, such as for defense against predation or infection, communication and socialization, life cycle development, and surviving environmental conditions. Arthropods harbor a large variety of chemical substances used for these ecological adaptations, and this is the overarching theme of the field of chemical ecology. Progress in the field has advanced rapidly, and this comprehensive review summarizes the enormous potential for discovery of new natural products with medicinal value from among the phylum Arthropoda. This review: (1) introduces the topic of arthropod chemical biodiversity; (2) reviews cultural uses of arthropods as medicines; (3) provides an overview of insect chemical defense studies and modern natural product analytical methods; (4) describes examples from the literature of insect-derived substances with medicinally relevant biological properties; and (5) summarizes the aforementioned topics to emphasize the value of arthropods as reservoirs of potentially useful new natural products.

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1 Introduction

The natural world is made of chemical compounds, and all life-forms on earth depend on efficient interactions between them for their existence. A host of technologies critical for human success, from the treatment of pathogenic diseases to food production to controlling pests, require an understanding of chemical interactions between organisms. Understanding the nature of the chemical and physical properties of substances and natural matrices is critical to our ability to isolate, characterize, produce, and use the chemistry of Nature to improve our livelihoods, and do so in a sustainable manner.

The field of pharmacognosy deals with the biological properties of natural substances that lend themselves to medicinal uses rooted in natural products chemistry. Harnessing the power of Nature's chemical laboratory, such as using substances (pheromones, *etc.*) that attract crop pests, and natural toxins that have the ability to kill cancer cells or microbial pathogens, involves drawing analogies from the ecological roles of chemicals to human applications. Even the natural mechanisms that organisms use to manufacture these substances – their biosynthetic pathways – have only recently been explored in depth, and only for a tiny fraction of species have potential applications been considered.

Chemical ecology is the study of how chemical substances mediate the interactions between organisms that give rise to the ecosystems we observe. Information derived from chemical ecology can be utilized in a 'biorational deduction' approach[1,2] to efficiently narrow down the search for the most promising medicinal chemicals from Nature.[1–9] One of the predominant forms of interaction in which organisms use chemicals is communication. Nearly all organisms, from bacteria to plants to insects to mammals, use chemicals to send signals to other organisms. Many of these substances are also used to protect the source organism from harm (predation, attack, infection, or simply invasion of living space). These substances can be broadly placed in the category of chemical defense. Toxins and other chemicals which harm or are offensive to various recipient organisms do so by producing a physiological response in the recipient/target. Such responses often have analogous counterparts relevant to human disease – such as toxicity to cancer cells or microbial pathogens.

Insects make up the largest and most diverse group of organisms on earth, making up 80–90% of the world's biodiversity.[7] Approximately 950,000 species of insects have been described,[10] with some authors estimating that there are approximately 4,000,000 insect species on earth in total.[11] Considering the sheer numbers of insect species, to say nothing of the diversity of niches they inhabit or the huge variety of ways they interact with other species and their environment through chemistry, it is clear that there are vast numbers of scientific and technological discoveries yet to be made in the world of these small armored creatures that surround us. The field of chemical ecology itself has been by far almost completely focused on insects, with the first pheromone (bombykol) having been discovered from the silk moth *Bombyx mori*.[12] Among the host of chemical tools insects use for survival and reproduction are a wide variety of chemical substances which they produce to ward off attack. For example, Pherobase©, the internet database of chemicals used for various types of communication (semiochemicals) from or involving mostly invertebrates, lists approximately 635 compounds as 'Defense Substances' (as of 23 July 2009).[13] However, only a small fraction of insect species on earth have been analyzed chemically or explored for the potential presence of medicinally relevant substances. In fact, little or nothing is known about the biosynthetic mechanisms or greater ecological significance of these chemicals in all but a handful of species.[14] Thus, studying the biosynthesis of various insect chemicals is likely to produce a wealth of useful information with applications in the fields of biochemistry, ecology, and biotechnology. For a variety of reasons, insects and their chemical defense systems present a valuable source of novel chemistry that certainly merits further investigation as source of new medicinal compounds, as well as substances with other applications.

This review introduces the topic of arthropod chemical biodiversity, describes examples of medicinal insect use by cultures worldwide, provides an overview of insect chemical defense studies and modern natural product analytical methods, describes examples of specific insect-derived compounds with medicinally relevant biological properties, and summarizes these topics to emphasize the value of arthropods as reservoirs of potentially useful new natural products. The literature on these topics is vast, but fragmented. Thus, this review amalgamates the most intriguing examples of cultural medicinal insect use and modern pharmacological studies of insect-derived substances to illustrate the potential that exists for drug discovery from arthropods, while acknowledging that it is not possible in a single review to cover all published insect chemical defense and medicinal insect studies.

Aaron T. Dossey

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2 Ethnoentomology: Use of insects by various cultures worldwide

Various cultures around the world, particularly in the tropics, have long histories of using insects for a wide variety of functions including production of materials such as silk, for art, in rituals, for food, and as medicines to alleviate disease and suffering.[2,15–33] Insects used as food is a topic which has been extensively reviewed, and is beyond the scope of this review.[19] A book called *Insecto-Theology: Or a Demonstration of the Being and Perfections of God* (F. C. Lesser, 1755), cited by Berenbaum[33] as being published in 1699, discussed the belief that insects exist for the benefit of human beings. Ancient texts refer to medicinal use of insects as far back as the 16th century BC (The Ebers Papyrus),[34] and the Chinese have used insects such as silkworms medicinally[35] for at least 3000 years.[2] Some insects are even utilized as tools. In a rather unique example, natives in Papua New Guinea use the spiked femurs of the stick insect *Eurycantha calcarata* as fishing hooks.[36] In another possibly more famous example, the Satere-Mawe people from the Amazonian basin of Brazil use bullet ants (*Paraponera clavata*) in rite-of-passage rituals for manhood and social status.[37–39] In this ritual, a hundred or so bullet ants are first mixed into a herbal brew until they are fully anaesthetized. Next, the ants are woven into a glove made of palm leaves with the stingers on their abdomens pointing toward the inside. The ritual must be repeated a total of 20 times in order to be respected as men by elders and hold leadership positions. The pain from even one of these stings can last several hours, and contains at least one peptide neurotoxin, poneratoxin (see also Sections 2.1 and 4.2).

A 2005 review by Costa-Neto[2] describes examples from the literature where other groups, such as the Arawak in Guiana, intentionally allow *Paraponera clavata* to sting their babies in order to stimulate them to walk early. Others allow this ant to sting in order to build resistance to future stings. Another tribe, the Ka'apor from the Brazilian state of Marahnão, use a related ant species (*Pachycondyla commutata*) in rituals similar to those described above using *P. clavata*, but where females undergo the stinging and the ants are woven into strings which are tied around the subject's forehead and chest.[2] Some tribes in South America apply poison from the skin of poison dart frogs (Family Dendrobatidae) to arrow tips that they use for hunting food. However, most of the alkaloids which are known to give these frogs their toxic properties are derived directly from their arthropod diets which are mostly made up of insects.[40–42] Thus even this use of a frog-skin secretion is actually an indirect use of insects.

Some of the above examples are cases of insect use by humans rooted partly in folklore tradition rather than medicinal application. Nonetheless, they are also examples of human use of natural products, derived from both insects and plants, to induce physiological responses, which may provide useful analogies to how the compounds involved might one day be used by drug discovery researchers.

Various natural sources of crude materials have been utilized by man to treat disease and discomfort for thousands of years. Research on the potential value these substances hold in modern medicine to provide remedies for human ailments has been the focus of several fields of study such as pharmacognosy, ethnobotany, ethnoentomology, entomotherapy,[2] and others. This section covers examples from the field of ethnoentomology, focusing on medicinal use of insects by various cultures worldwide. However, it should be noted that many of the older medicinal remedies used by various cultures in general do not actually work, or have not yet been tested or verified using rigorous modern biomedical studies, and thus may or may not hold merit. Nonetheless, it is worth noting and discussing here the various ways in which insects have been utilized as medicines in different cultures, especially in more modern traditional Chinese medicines, and that some of the examples may actually be valuable leads in the development of a useful drug. Later in this review, examples from more modern studies designed to determine the actual medicinal properties of isolated chemical components from insects and other arthropods will be explored.

Historically, traditional use of plants as medicines, known as 'ethnobotany', has been extensively recognized, studied, and reviewed.[43] However, such attention has not been paid to the potential of arthropods as a source of medicinally relevant substances.[44] Trowell points out that there are at least 16 times as many insect species as there are plant species, yet plant chemistry has been studied 7000 times as much as insect chemistry when comparing the amount of research per species.[45] Phytochemical studies have indeed demonstrated the value of plants in providing a large number of biologically active natural products.[46] There are important reasons for this, such as: (1) plants have historically been utilized much more than insects for food and medicine by most cultures, and thus the physiological effects of plants on humans have been more frequently encountered throughout history, and (2) plants essentially 'start from scratch' in their chemical production by biosynthesizing most of their compounds *de novo* from carbon dioxide, water, elemental oxygen, and very simple nitrogen sources, and thus are largely non-dependent on precursors which they must ingest from other organisms. Nonetheless, the vast biodiversity which exists in the arthropod world, compared to all other organisms on earth, certainly suggests that arthropods should be given a more serious look. In fact, insects and plants often utilize similar toxic chemical compounds for defense, and studies of these compounds in plants provides a framework for these studies in insects.[47] Additionally, many past and present cultures prescribe a variety of preparations of insects and insect-produced materials to alleviate disease and suffering.

Entomotherapy is the term used to describe the use of insects for medicinal purposes. So far, one very good comprehensive review has been written on this subject by Costa-Neto.[2] His review covers literature on cultural holistic use of insects by various cultures worldwide up to the end of 2005. In that review, he tabulates at least 64 different terrestrial and aquatic arthropod species from at least 14 orders and 3 classes, including insects, arachnids, and centipedes, all prescribed for medicinal uses by various cultures from 5 continents. He also cites an estimated commercial value of insect-related products to be over US$100 million.[17] Costa-Neto has also written articles focusing on medicinal use of insects and other animals in Brazil.[2,17,18,48] These articles focus primarily on cultural and holistic uses of insects. Additionally, an article by Yoo *et al.* cites 7 different studies in which 7 different insect species were used to treat cancer and other diseases.[49] Several of the more intriguing examples of medicinal insect use are described here, along with additional examples, and pharmacognosy or biorationalization-based examinations of those examples.

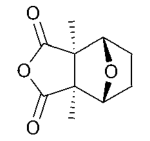
Most medicinal uses of insects by various cultures involves some preparation of the whole insect, and thus it is often unclear whether the active components (if the preparation is indeed efficacious against disease) are derived from the chemical defense system or other part of the insect or its gut contents. However, in many of these preparations of insects for medicinal use the insects are roasted or fried, which may destroy any medicinal properties they may have had. Nonetheless, the active principles in these remedies, if they exist, may actually be released, created, or at the very least minimally harmed by the cooking process and still may be contained in the final elixir. Additionally, many medicinal insect preparations do not involve heating or cooking. Thus, several examples warrant mention here, since many insect compounds, such as those utilized in their chemical defense systems, have evolved to have physiological effects on vertebrates, including humans. Additionally, to date there have been relatively few articles covering the topic of medicinal use of insects,[2,16–18,22–25,27–35,39,48,50–54] and only one that is geographically comprehensive.[2] Nonetheless, some very good studies, which are reviewed in this section, have been produced which outline a large number of examples of entomotherapy by cultures in different parts of the world.

2.1 Latin America

Use of insects in medicines has been well documented for native peoples in Latin America.[2,17,18,27,28,48,54] Recently, a report by Ferreira *et al.* described the use of 8 insect species, among a total of 31 different animals, which are sold for medicinal purposes in markets in Crato and Juazeiro do Norte in the state of Ceará, Brazil.[54] In the most recent and comprehensive review,[17] Costa-Neto notes that medicinal use of insects has been reported in at least 13 Brazilian states since colonial times in over 16 different reports.[2,17] In a 2002 report, Costa-Neto documents medicinal use of at least 42 insect species from 9 orders used in the northwest Brazilian state of Bahia.[17] Many of the insect-derived concoctions are used in the form of teas made from boiled (or toasted and boiled) insects or insect powder. One example described by Costa-Neto[17] is the common house fly (vernacular name '*mosca*'; *Musca domestica*) in Tanquinho, Brazil, to treat furuncles (or 'boils', a skin infection caused by microbial parasites such as *Staphylococcus aureus*). The flies are prepared by crushing them and applying the resulting paste directly onto the infected area.[17] It is worth noting that an antibiotic-resistant strain of *S. aureus* (MRSA, methicillin-resistant *Staphylococcus aureus*) is one of the major examples of an emerging pathogen in need of new antibiotics. Another medicinal insect reported in the same article are ants from the previously mentioned genus *Paraponera* ('Large Stinging Ant'; local name '*formiga-preta-grande*' or '*formigão*'). The sting of these ants is used by people from Feira de Santana, Brazil, to treat rheumatism and backaches. The venom from these ants, known to possess the most painful stings of any ant in the world, contains a potent peptide neurotoxin called poneratoxin, which may be amenable to pharmacological adaptation as a painkiller.

The venom of other Hymenoptera such as honeybees (*Apis* sp.) is used commonly worldwide for treating rheumatism, arthritis, and other related conditions (see also discussion of mellitin and apitherapy in Section 4.5). At least 22 types of insects in the order Hymenoptera (ants, bees, and wasps) were described by Costa-Neto as being used by people in Bahia for various medicinal purposes.[17] In fact, the stings of honeybees are commonly used in many cultures, including the United States, to treat rheumatism and arthritis. The honey from at least 5 genera of bees (*Apis*, *Melipona*, *Partamona*, *Plebeia*, *Tetragonisca*) is used in several localities in Bahia to treat ailments such as cold, flu, tuberculosis, and sore throats, as well as in a topical application for the treatment of burns, among other things.[17] In fact, the mixture of honey and lemon juice continues to be used by Western cultures as a common remedy for colds, flu, and sore throats. Honey has been shown to possess antibacterial properties,[55,56] which could help reduce the effects of primary or secondary infections. It has also been suggested as a valid wound-dressing agent in a modern study by Efem *et al.*[56] In addition to honey, several studies have verified antimicrobial activity in other bee products such as propolis (a waxy substance which bees use to seal their nests)[57,58] – see Section 3.4.

Some people in Bahia state, Brazil, were observed using a tea made from a blood-sucking bug in the genus *Triatoma* to treat a variety of ailments.[2] Also, some people use masses of crushed stink bugs (family Pentatomidae) placed near the nose to unblock sinus congestion. This could have some legitimate basis, since insects such as these are known to produce a variety of different volatile compounds (pheromones, defensive compounds, *etc.*),[59] some of which may reduce inflammation – however, this hypothesis requires experimental verification. Some insects are even prescribed for impotence in Brazil. For example, the sting of an ant known as '*formigão*' (*Dinoponera* sp.) is allowed to 'sting the gland' for 'strengthening a flaccid penis'.[2] Presumably, inflammation from the venom toxins causes penile swelling in this case, similar to the primary mode of action for cantharidin (**1**, Fig. 1), a defensive substance from blister beetles which is discussed in greater detail in Sections 2.3 and 4.1. Of course, other more medicinally efficacious/promising modes of action are possible, and such hypotheses need to be verified experimentally. Also, the non-native darkling beetle *Palembus dermestoides* (order Coleoptera, family Tenibrionidae) is used in Brazil to treat sexual impotence as well as asthma, tuberculosis, and arthritis.[2] Other people in Brazil, known as the Zuruaha, use the sting of an ant that they call '*takarisi*' as an analgesic.[2] If this remedy is effective, it is almost certainly due to the presence of neurotoxic substances, and definitely worth further investigation.



1

Fig. 1 Cantharidin (**1**), the major active blistering component from defensive secretions (blood/hemolymph) of blister beetles and the well-known concoction made from those beetles called 'Spanish Fly'.

In addition to the use of medicinal insects by Brazilians, ancient Mexican cultures have been reported to use a number of different insects to treat disease and injury.[27] For example, the Maya are reported to have used maggots for therapeutic purposes 1000 years ago.[17] Ramos-Elorduy *et al.* describe the use of 18 insect species by Mexican indigenous groups to treat several illnesses such as goiter, tuberculosis, whooping cough, cough, cutaneous eruptions, and liver, stomach, and kidney problems.[2,60] In a 1988 paper by Ramos-Elorduy *et al.*, 43 species of insects (from 16 families and 6 orders) were noted as being used for traditional medicine by both ancient (Aztecs and others) and modern Mexican cultures.[27] They solicited information from over 9 modern Mexican cultures and collected insect material from several states in Mexico. These authors note that some medicinal insect use by Mexican cultures appeared to be allied with the 'Doctrine of Principles' whereby the plant or animal used (or its part) simply resembles the part of the body being treated. These examples are unlikely to hold much medicinal value. Among the medicinal uses of insects observed by those authors, some of the more intriguing examples and their potential implications are described below.

In one example, oil from several species of true bugs (order Hemiptera) was applied to the skin for the treatment of scrofula and other skin diseases often caused by *Mycobacterium tuberculosis*, the causative agent of tuberculosis. It is also used as an analgesic and anesthetic. Another medicinal insect example cited was the mealybug (*Coccus axin*, local name '*aje*'). This insect produces a waxy secretion and is used as an ointment. The insects are boiled to produce a sticky mass which his placed over lesions from leprosy, burns, and other skin conditions. They are also used to treat diarrhea or to clean teeth. This sticky mass could simply prevent desiccation of exposed wounds, or it could contain antimicrobial properties that might warrant more rigorous investigation. Another insect, a beetle from the family Meloidae referred to in the Nahuatl language as '*tetl ocuillin*' (translated as 'fire worm'), is used as an aphrodisiac and to treat urogenital disorders. As previously discussed, the aphrodisiac properties of these beetles (such as in the medicinal preparation called 'Spanish Fly') are likely without merit, but a patient's urogenital system might possibly benefit from the effects of cantharidin in other ways. Several ants and other Hymenoptera were cited in the report as being used for medicinal purposes in Mexico. The decapitated heads and mandibles of one of these, the leafcutter ant (*Atta* sp.), were used to close wounds after surgery. This is a common practice throughout South America. Ramos-Elorduy *et al.* report that the mandibular secretions of these insects may also have antibiotic properties and prevent wound infection, which is a plausible hypothesis. In fact, such ant-derived antimicrobial protection may actually come from the ants' associated microbial flora.[61] As a final example, the sting of an ant in the genus *Pogonomymex* from Mexico is used to treat rheumatism, arthritis, and poliomyelitis. For reasons previously mentioned for other medicinal ant and bee stings, the value of this therapy for treating rheumatism and arthritis is likely due to its anti-inflammatory properties.

2.2 Africa

Medicinal insect use, as well as common use of insects as food, has also been documented in Africa.[2,24,62–65] Overall, published examples of medicinal use of insects seem to be much less common for Africa, as well as Europe and North America, than for Asia and South America. An article by Nonaka documents such examples from the central Kalahari San people demonstrating various uses of insects for many functions, including as medicine, in hunting, for beauty, for decoration, as items for 'children's play', and especially as food.[24] One medicinal insect described in the article is the bagworm (family Psychidae, local name '*k'aarí*'), whose body juices are applied topically to treat stomatitis (a general term for inflammation of the mucus membrane of the mouth, which can be caused by infection or other conditions). If this treatment is effective, it could therefore be due to antimicrobial or anti-inflammatory agents in the bagworms.[24]

The aforementioned review by Costa-Neto[2] also summarizes some examples of medicinal use of insects by African cultures. For example, in southwestern Nigeria, the gut contents of mole crickets (*Grullotalpa africana*) are smeared on the feet to ward off foot infections.[63] This example may have merit, since insects are known to contain antimicrobial substances, as discussed in section 4.3. Additionally, insects are known to harbor a large number of microflora, particularly in their gut. Microbes are constantly in a state of chemical warfare with other microbes, so the gut contents of insects may also contain microbe-derived antimicrobial substances that could be beneficial for killing off infections. A report by Mbata describes how five classes of arthropods (Insecta, Arachnida, Crustacea, Diplopoda, and Chilopoda), including ten orders of insects, are used medicinally in Zambia.[2,64] For example, six species of cockroaches (order Blattaria) are used to treat boils (caused by infected hair follicles) and other wounds. Cockroaches, like many other insects, tend to live in 'dirty' microbe-rich environments such as soil and decaying matter. It is thus highly likely that they harbor antimicrobial substances, either self-generated or derived from their associated microflora, and this may possibly be the reason for any efficacy this remedy may have.

In Zaire, Antonio reported folk uses of at least 18 different insects.[62] In one example from that report, the 'trembling red ant', known locally as '*nkaam*', is used to treat bronchitis (locally referred to as '*muyeem*'). The ants, which reportedly produce 'sticky saliva', are placed in a bowl with water and given to the patient. This is repeated for one week. The merits of this example may lie in either antimicrobial or anti-inflammatory substances in the ants, possibly from their venom. A second intriguing example is the use of a bee (local name '*ngobo*') as a remedy for 'shuttering'. For this treatment, a healer places several bees in a container of wine, which the afflicted individual then drinks. It is possible that potent neurotoxins released from the bees' venom result in the effect of this concoction, or that alcohol or other components in the wine are the active principle. A third and final example from this report that appeared to have some potential merit for further investigation is the use of the tsetse fly (local name: '*kebty*'). This fly is famously known for being the main vector for trypanosomes (protozoan microorganisms) which cause 'African trypanosomiasis' or 'sleeping sickness'. After a patient has been bitten by one of these flies, the fly is crushed and rubbed onto the skin, and an incision is made at that point. This treatment is supposed to protect the fly-bitten person from sleeping sickness.[62] Considering how vaccines were discovered and function, it is plausible that this treatment actually functions as a rudimentary vaccine.

2.3 Asia

In addition to native peoples in South America and Africa, several examples of insect use in traditional European and Asian medicine exist in the literature.[2,20,25,26,28,33,35,51,66,67] Asian medicine continues to use various exotic ingredients such as insects even today, which remains one of the distinct differences between East Asian and Western medicine.[26] A translation of *Chinese Materia Medica* by Read (itself a translation of the *Pen Ts'ao Kang Mu*, an encyclopedia of traditional Chinese Medicines from circa 1596), gives examples of medicinal arthropods from Chinese culture, including bee and silkworm products, insect excreta and galls, hornets, wasps, bees, mantises (and their egg cases, or oothecae), flies, stink bugs, caterpillars, beetles, butterflies, cicadas, mole crickets, silverfish, cockroaches, dragonflies, locusts, lice, spiders, and scorpions.[30,51,68,69] According to Huang, the *Shennong Pharmacopoeia* (100–200 AD) describes 21 medicinal insects, and at least 52 additional examples were provided in *Compendium Materia Medica* (1578).[67]

Probably the most famous historical example of an insect used in both European and Chinese medicine is that of 'Spanish Fly'. This medicine originally came from the blister beetle *Lytta vesicatoria* (family Meloidae). The hemolymph (blood) of these insects contains a potent blistering agent (vesicant), cantharidin (**1**). When disturbed, many blister beetles exude droplets of this blistering blood out of pores in their leg joints so that it rubs off on the attacker (Fig. 2). Blister beetles have been used by the Chinese for removing warts and cancer treatment[22,70] and by the Greeks for enhancing sexual libido.[22] Spanish Fly is currently illegal in the United States except for use in the treatment of warts or in animal husbandry.[50] The use of Spanish Fly for enhancing sexual performance is largely warrantless; it simply causes irritation of the urinary tract which gives a false impression of sexual stimulation.[29] However, other medicinal applications of cantharidin may have some merit – see Section 4.1 for more details.

Chinese culture, both ancient and modern, is well known for its use of natural materials and remedies. The Chinese continue

Fig. 2 A blister beetle (*Epicauda* sp., family Meloidae) deploying its typical defensive secretion of blood (hemolymph) enriched in the blistering agent cantharidin (**1**). Photograph by Aaron T. Dossey. Genus identification by Michael C. Thomas.

to be the leading producers of insect-derived products such as silk, insect wax, and Chinese gallnuts. Insects are also utilized in China for a number of industrial processes including mass production of drugs and pesticides.[32] Besides the aforementioned blister beetles, many other insects have been and continue to be used in traditional Chinese medicine.[2,20,25,26,28,32,35,51,66,67] Cockroaches (order Blattaria) such as *Eupolyphaga sinensis* and *Opisthoplatia orientalis* are mass-produced in China and used for 'traumatic and vulnerary' medicines and in some other health products.[32,67] Tablets and other preparations of *Eupolyphaga* species, as well as other insect-derived medicines, can currently be obtained from a variety of commercial sources on the internet and local Chinese medicine markets. Other cockroaches are also used 'for internal feverish chills'.[30] In another study by Namba *et al.*, 54 different uses of medicinal insects were identified in the *Jing Shi Zheng Lei Da Guang Ben Cao*, a traditional Chinese medical text written during the Song Dynasty (1280 AD).[23] In general, there have been many scientific articles published in Chinese which give examples of medicinal properties of insects used in Chinese medicine. Unfortunately, many of these examples are published in Chinese only, and thus were not amenable to examination for this review.

Costa-Neto has also reviewed literature examples of medicinal insect use by the Chinese.[2] In one very intriguing example, Chinese people living in Malaysia raise stick insects (order Phasmatodea; phasmid chemical defense is also discussed in Section 3.2) for the medicinal use of their feces (or frass, to use the technical term). This frass is dried and mixed with herbs to treat asthma, upset stomach, and muscle pain.[2,30,71] It is very possible that any therapeutic effect from this frass comes from the plants on which these insects feed. One might ask: 'Why not simply use the plants?' It is also possible, however, that the insects and/or their internal microflora process and/or sequester compounds from the plants, resulting in material that is far more effective than the host plant itself. Zimian *et al.* reported that 15 arthropods are currently used in Chinese medicine on a regular basis, including the following species which have been the subject of pharmacological studies: *Malaphis chinensis*, *Bombyx mori*, *Hepialus armoricanus*, *Mylabris cichori*, and *Buthus martensii* (a scorpion).[2,35]

Ants are used extensively in Chinese medicine. One of the most common examples is the 'weaver ant' (*Polyrhachis vicina*), which is used in an elixir (sometimes called 'Chinese Mountain Ant extract') commercially available on the internet. In fact, the weaver ant is used so extensively that the species is threatened with extinction due at least in part to over-harvesting.[2,17,72]

Additional examples of medicinal insect use were reported by Chen in 1994.[73] Chen noted that ants used as medicines have been reported in over 200 newspapers and magazines, and continuously on Chinese television and radio stations. In that article Chen also reports an estimate provided by the Ant Therapy Center in Nanjing that "at least 5000 pounds of clean ants are consumed each year". Chen's article focuses on Chinese use of ants as both food and medicine.[73] A study by Zhang *et al.* reports that pain was alleviated in mice by feeding them a powder made from dried weaver ants (11.25 g of ant powder per kg of mouse weight).[73,74] Another study by Zhao *et al.* reported that weaver ant paste could provide liver protection in rats suffering from chronic hepatitis. In that study rats fed with the ant paste (2.4 g/kg) had a reduction in the level of guanosine triphosphate (GTP)[73,75] which is needed for viral RNA synthesis.[76] In another report, it was noted that treatment with ants improved the health of mice with cancer by increasing their appetite, relieving pain, and increasing white blood cell levels.[73,77]

Other Asian cultures have also incorporated insects into their medicinal traditions. For example, the Mamusi people of Papua New Guinea reportedly use the actinidine-containing defensive spray of the stick insect *Megacrania nigrosulfurea* as a topical treatment for ulcers.[78] Additionally, in 1999 Pemberton reviewed the extensive use of arthropods in Korean medicine.[26] South Korea hosts one of the largest traditional medicine markets called the '*Kyeong Dong Shijang*' which is comprised of over 900 businesses. In his review, Pemberton collected information from interviews with various Korean doctors in this large market area to determine the extent of use for various medicinal arthropods listed in the Korean language 'Illustrated Natural Drugs Encyclopedia'. Among the 19 different examples tabulated, centipedes in the genus *Scolopendra* and silkworm larvae (*Bombyx mori*) infected with an entomopathogenic fungus (*Beauveria bassiana*) were two of the three most used medicines. Centipedes were used to treat joint problems, lumps or masses, poisonous tumors or carbuncles, and neoplasms among other things. It is unclear if centipedes are actually effective medicine against any of these diseases. However, centipedes are known to be venomous, and thus may contain pharmacologically relevant substances. Additionally, scorpions (*Buthus martensis*) were another example of a known toxic animal highly used in the clinics interviewed. Silkworm larvae infected with a fungus, one of the other most used sources of medicine described in Pemberton's review, were used to treat stroke, tonsillitis, and rubella among other diseases, while adult male silkworm moths were used to treat impotence and premature ejaculation. Additionally, the frass of silkworm larvae was also used to treat diabetes, neuralgia, and skin rash, among other things. This remedy for diabetes may have some merit, as a product made from silkworm larvae called 'silkworm powder' is also sold in Asia as a treatment for the disease. See Section 4.5 for a discussion of compounds from mulberry (*Morus* sp.), the foodplant of *Bombyx mori*.

The introduction to an article by Yoo *et al.*[49] cites several studies in which larvae (or grubs) of the flower beetle *Protaetia brevitarsis* (called '*jejo*' in Korea) (order Coleoptera, family Scarabaeidae, subfamily Cetoniidae) has been used to treat hepatic cancer, liver cirrhosis, hepatitis, breast cancer and inflammatory disease. Even chitin, the major component of arthropod exoskeletons, has been used in Korean medicine for wound healing. Chitin is a linear polysaccharide, a β-1,4-linked polymer of *N*-acetyl-D-glucosamine (GlcANc), which is also found in the cell walls of insects and fungi.[79] The source of chitin used in Korean medicine is referred to as 'the pen of an octopus',[80] but it is likely that insect chitin possesses the same properties.

Traditional use of insects as medicines has also been practiced in India.[2,25,65,81] Oudhia has written extensively on various examples of insects and other arthropods with medicinal uses by various communities in India.[25] Over 500 species of insects and arachnids (mites and spiders) are used by over 3500 healers for a variety of medicinal purposes in the Indian state of Chhattisgarh alone.[2,25] For example, oil from the 'red velvet mite' (*Trombidium grandissimum*) is used to treat at least 10 different diseases, including malaria urogenital disorders, and paralysis.[2,25] These mites are soil dwellers, and thus are likely to contain antimicrobial substances to protect them from soil-borne pathogens and might also be effective against Malaria. The 'pod borer' or 'gram caterpillar' (*Helicoverpa armigera*, also known in the United States as cotton bollworm, American bollworm, corn earworm, tobacco budworm or old world bollworm) is widely distributed in India, USA, and other countries, and is a major pest of economically important crops such as corn, cotton, sorghum, and other cultivated plants.[25] This moth is reportedly used in several Indian villages in medicine,[25] being used alone and in combination with some herbs. Villagers use the powder of the dried and crushed insects in tonics to treat fevers, general weakness, and nervous breakdown. Fresh extracts of the caterpillars are also applied to injuries to stop bleeding. Such uses of insects may hold merit in wound treatment due to antimicrobial substances they might contain.

Some arthropods (such as those which feed on blood) are also known to contain anticoagulant substances. Crushed insects also tend to be sticky and harden in the open air, which may serve to seal wounds. One healer was reported to use aqueous extracts of the 'pod borer' moth, applied externally, to promote hair growth. In India, *Helicoverpa armigera* is used to treat over 50 different diseases. Thus, it is a very good example of how a major pest might be utilized for beneficial purposes. In fact, many insects occur or can be cultivated in large numbers. Accordingly, pest species in particular are a major reservoir of material for study, and thus represent 'low-hanging fruit' for natural product prospecting. Another insect, the 'green leaf-hopper' (*Nephotettix nigropictus*), is used to treat diseases such as gonorrhea (a sexually transmitted microbial infection caused by the bacterium *Neisseria gonorrhoeae*). In this case, as with the pod borer, freshly crushed green leaf-hoppers are applied to the affected area. Antimicrobial substances in the insects may be the active ingredient in this remedy. Additionally, the desiccating properties of drying insect hemolymph may be effective in killing microbes when applied topically. As a final example from the report by Oudhia, lightning beetles (also known as fireflies; order Coleoptera, family Lampyridae) are crushed fresh and applied to wounds to stop bleeding. These insects may contain antimicrobial substances which help with wound healing, or the drying insect matter may help seal the wounds and starve the microbes of water. Of course such hypotheses require further investigation. In fact, at the end of his report, Oudhia states that "These surveys suggest that … there is a need for more extensive surveys" and that "identification of potential formulations and systematic clinical trials are essential".[25]

In addition to the reports by Oudhia, others have cited medicinal use of insects in India. For example, Sharma *et al.* reported medicinal insect use by nine tribal communities in Rajasthan.[2,81] This report included 10 species of invertebrates (including insects and crustaceans). It described which parts/secretions from the animals were used, how they were used, and what they were used for.[2,81]

2.4 Europe

In Europe fewer examples of medicinal insects have been described than from other regions of the world.[28,33] However, the aforementioned use of cantharidin (**1**) for a number of medicinal purposes was described as early as 50–100 AD,[22] and became popular in France as an aphrodisiac in the late 1700s.[22,82] Ratcliffe[28] cites the topical application of oil obtained from the May beetle *Melolontha vulcaris* to treat scratches and other wounds, as well as for rheumatism. The adult beetles of that species were also soaked in wine and used to treat anemia.[2,28,33] These types of beetles, both in their adult and larval stages, live in the soil and the larvae feed on rotting plant matter. Thus, in this microbe/pathogen-rich environment, it is logical that their bodies contain a number of antimicrobial substances that might be beneficial in wound healing. In eastern Europe, propolis from bees is used as an antiseptic and anti-inflammatory agent for wounds.[2,83] Such a treatment may also benefit from antimicrobial properties of propolis[84] (see also Section 4.3).

3 Examples of chemical biodiversity in insect chemical defenses

Insects and other arthropods use a wide variety of chemical substances to defend themselves from attack and infection.[44,59,85–92] Insect chemical defenses have intrigued scientists for over 100 years, particularly in the past half-century.[86,87,91,93–96] Even before the technologies required to analyze the underlying chemistry of these creatures existed, descriptions were being made of how insects defend themselves from predators in Nature[92,95,96] and of the anatomical features[93,95,96] of their chemical weapon machinery. The topic of insect chemical defense has been reviewed in several very good articles,[59,85,88,90–92,97] books,[86,87,89,98] and chapters.[44,82,99] Specifically, the chemical makeup and functions of several types of insects (including ants, bees, and wasps (order Hymenoptera),[89,98,100,101] beetles (order Coleoptera);[85,97] and true bugs (Order Hemiptera);[59]) have been extensively reviewed. Thus, only a brief overview of historical context, technological advances, and case examples of chemical diversity in two insect orders are given here.

3.1 Insect chemical defense research: Technology and history

With the discovery of the first pheromone from the silk moth *Bombyx mori* by Butenandt *et al.* in 1961,[12] scientists have been studying the molecular makeup of the chemical language utilized by insects and the ammunition deployed in their chemical defense systems.[6,44,86,87,92,94,102] Rational scientific studies discovering and utilizing the principles of natural products chemistry were a hallmark of scientific inquiry for the entire 20th century, and continue today. This era was characterized by the development and subsequent utilization of modern analytical chemistry employing such important techniques as chemical extraction,[103] chromatography,[103–105] mass spectrometry,[105] NMR spectroscopy,[105–108] and others.[109] These methods, particularly extraction and isolation, utilized the principles of natural products chemistry, pharmacognosy, and chemical ecology to isolate, identify, and demonstrate the function of thousands of natural compounds in both their ecological niches as well as medicinal and other applied contexts. In fact, what makes these newer techniques particularly valuable to the much-needed increase in natural product exploration is their ability to identify biologically active substances from ever-smaller amounts[106–108,110] of material and/or from mixtures,[109] or both. For example, work by Gronquist, Meinwald, Eisner and Schroder have demonstrated methodology which allowed them to identify 13 new steroid structures from an extract of just 50 individual fireflies (*Lucidota atra*, order Coleoptera).[111] These and other recent advances bring within reach previously intangible new sources of natural products from species which can be in small supply, including invertebrates such as insects and other arthropods. Nonetheless, some aspects of the art of sample handling and preparation remain a very human endeavor, with carefulness and efficiency being paramount to success. Thus, it is even more imperative that thought be put into how to isolate crude natural matrices most efficiently to obtain the purest and most concentrated source of the target material. For example, the stick insect research from my own work which is described below depends largely on collecting only the exuded secretions from those creatures, rather than whole-body or tissue extracts. Understanding the source of the desired material is very important when handling small samples. Today major recent advances in all of the standard analytical chemistry techniques (NMR, mass spectrometry, and chromatography) have created an environment where chemical ecology and natural products chemistry are now better equipped than ever to capitalize on new and promising natural reservoirs of useful compounds.[4–6,8,9,106–108,110,112] Outfitted with the latest in analytical capabilities, high-throughput biological activity screening methodologies,[113] and expanded knowledge of the planet's biodiversity, chemical ecology is well placed to substantially inform and guide natural products and pharmacognosy researchers to new and better drugs.

Insect chemical defenses are particularly attractive for studies seeking biologically active natural products. Since the late 1950s, the famous 'dymanic duo' of Eisner and Meinwald at at Cornell University have pioneered many seminal studies characterizing the chemistry of insect chemical defense systems in a host of species from many different orders as well as non-insect arthropods such as millipedes (class Diplopoda) and others.[86–88,114] Much of the pioneering work that initiated the field of Chemical Ecology and arthropod chemical defense research stemmed from the work of these two scientists. Some of Eisner and Meinwald's earliest work included isolation and characterization of chemical defense compounds sprayed from insects such as beetles and stick insects. Probably their most famous work focused on the chemical defense of the bombardier beetle and the mechanism of how this beetle ejects an explosive toxic secretion at high temperature when threatened.[86,87] Eisner has published two books which summarize several of the more prominent examples of insect chemical defenses which he and Meinwald have elucidated: *For the Love of Insects* and *Secret Weapons: Defenses of Insects, Spiders, Scorpions, and Other Many-Legged Creatures*.[86,87] Eisner and Meinwald also co-edited *Chemical Ecology: The Chemistry of Biotic Interaction*, a collection of chapters on chemical ecology by some of the top scientists in the field.[6]

Other pioneers in the field of insect defenses have included Blum, Schildknecht, Pavan and Pasteels, among many others.[44,87,92] Blum published the first book ever dedicated to the topic of chemical defenses in insects and related arthropods entitled *Chemical Defenses of Arthropods*.[89] That book provides detailed information on chemical structures as well as behavioral aspects of the chemical defense systems utilized by many species. Blum himself has made countless major contributions to the field of chemical ecology as a whole.

3.2 Order Phasmatodea (stick insects)

Stick insects (walkingstick insects, or phasmids) make up a rather small order of insects, having just over 3000 species.[115–119] They are most famous for their non-chemical defense: camouflage.[120] Their common name, stick insect, indicates how some species avoid predators by blending into their environment due to their resemblance to sticks, twigs, or leaves. However, the more prominent defense mechanism for many phasmid species is not their ability to hide, but for the irritating chemical substances

Fig. 3 (A) A mating pair of the black-and-white (Ocala National Forest) color form of the stick insect *Anisomorpha buprestoides* deploying its chemical defense spray (females are larger than males). (B) *A. buprestoides* successfully repelling a brown Cuban anole (*Anolis sagrei sagrei*), a non-native invasive species in Florida. These still images were taken from a high-speed video recorded by Rod C. Clarke and Adam Scott for the 'Insects' episode of the BBC television series *Life*.[150]

which they deploy against attackers, predators, or anyone unfortunate enough to get too close (Fig. 3).[94,95,110,112,119,121–144] In fact, many species of stick insects do not blend into their background well at all. These species are brightly colored as a warning to predators (aposematism).

Though the Phasmatodea contains relatively few species when compared to other insect orders, they possess a relatively high level of compound diversity, originating (in all species studied so far) from the same pair of glands. Many species possess a pair of prothoracic glands which have evolved to produce and release material, either a visible liquid or vapor, when disturbed. These very specialized glands tend to produce very specific compounds in quite pure form, which are often quite easy to collect (Fig. 4).

The chemical makeup of defense sprays from very few of the more than 3000 named species have been published to date. However, among those 11 species, at least 25 compounds have been identified so far (Fig. 5).[94,110,121,123,129,131–133,135,137,139–141,143] Given the number of phasmid species analyzed, the number of compounds found so far from them, the number of novel compounds found in phasmids so far, and the total number of species in this order, phasmids represent a significant potential source of new compounds. Additionally, some phasmids are the largest chemically defended insects in the world, and they live much longer than many other insects. Also, phasmids are kept as pets by a large number of people worldwide, especially in Europe.[120,130] Considering their size, ease of raising in captivity, possession of very specialized defense glands, and the variety of chemicals that a few species are known to produce, these insects represent an intriguing model system in which to study defensive chemical biosynthetic pathways in insects.

Phasmid chemical defenses have been the topic of various studies for well over 100 years, the defense glands of phasmids having been described as early as 1876 by Scudder.[95] In 1895, Packard[96] described the structures and functions of various arthropod 'eversible repugnatorial scent glands', noting that they are predominantly used by terrestrial arthropods and absent in nearly all marine arthropods. The first publication which described the chemical analysis of a phasmid defense spray was conducted by Schneider in 1934 on the Chilean species *Agathemera crassa* (referred to previously as *Paradoxomorpha crassa*).[94] However, the structure (**2**) reported in that study is impossible, and it seems probable that the correct structure is that determined for the defense compound of

Fig. 4 The author (Dr. Aaron T. Dossey) milking an adult female of *Anisomorpha buprestoides* (Ocala National Forest color form). This still image was taken from a high-speed video recorded by Rod C. Clarke and Adam Scott for the 'Insects' episode of the BBC television series *Life*.[150]

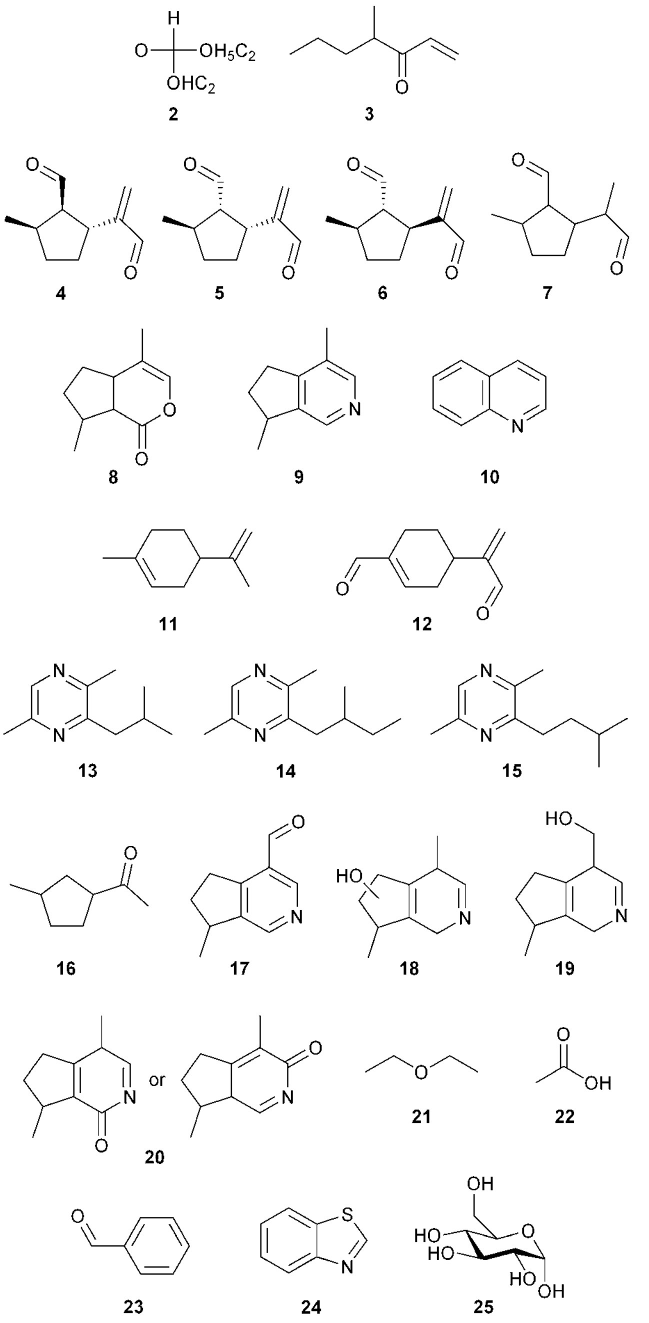


Fig. 5 Compounds from the defense glands of phasmids.

another species in that genus (*Agathemera elegans*), which was more recently determined to be 4-methyl-1-hepten-3-one (**3**), reportedly a novel natural product not previously identified from any other natural source.[140] Subsequent to the 1934 publication by Schneider, the next phasmid studied was *Anisomorpha buprestoides*. The defensive spray of that species was characterized both chemically[134] and ecologically[139,145] in classic works by Eisner, Meinwald, and coworkers.[134,139] The medical literature also provides examples of both human and dog encounters with the chemical weaponry of this species, noting the intense pain caused when the spray comes into contact with the eye.[146–148]

Anisomorpha buprestoides (also known as the 'devil rider', 'musk mare', 'prairie alligator', or the 'two-striped walkingstick insect')[142,149,150] produces a monoterpene which was first identified by Meinwald *et al.* in 1962 and named anisomorphal (**4**).[139] Subsequently, my collaborators and I have shown that this species produces three diastereomers of anisomorphal (anisomorphal (**4**), dolichodial (**5**), and peruphasmal (**6**)) which vary in relative concentration between different developmental stages and populations when individual defense secretions are samples and analyzed.[110,112,121,130,133,151,152] In general, the various phasmid species analyzed to date have been shown to produce a rather wide variety of compounds (Fig. 5), including: methyl-1-hepten-3-one (**3**) from *A. elegans*;[140] anisomorphal (**4**) from adult *A. buprestoides*;[121,135,139] dolichodial (**5**) found in young *A. buprestoides*;[110,112,133] peruphasmal (**6**) from *Peruphasma schultei* and young, as well as some wild adult populations of *A. buprestoides*;[110,112,121,133,153] iridodial (**7**) from *Graeffea crouani*;[141] nepetalactone (**8**) from *Graeffea crouani*;[141] actinidine (**9**) from *Megacrania tsudai* (misidentified as *M. alpheus*)[129,137,154] and *M. nigrosulfurea*[78]), quinoline (**10**) from *Oreophoetes peruana*,[135] limonene (**11**) from *Sipyloidea sipylus*;[123] parectadial (**12**), only known from the phasmid *Parectatosoma mocquerysi*;[132,138,143] and alkyldimethyl pyrazines **13–15** from *Phyllium westwoodii*;[131] and others as minor components – 1-acetyl-3-methylcyclopentane (**16**) and analogs **17–20** of actinidine (**9**) from *Megacrania tsudai*;[137] and diethyl ether (**21**), acetic acid (**22**), benzaldehyde (**23**) and benzothiazole (**24**) from *Sipyloidea sipylus*.[123] One of the most exciting recent discoveries in stick insect defensive chemistry is that of a novel compound, parectadial (**12**) from the species *Parectatosoma mocquerysi* of Madagascar.[132,138,143] The defensive secretion from *P. mocquerysi* is reported to cause reddening and peeling of the skin, but no pain or irritation (Fig. 6).[132]

The effects of parectadial (**12**) on human skin suggests that it may possess some pharmacologically useful properties. Perillyl alcohol and perillylaldehyde (both of which have the same carbon skeleton as parectadial, but a different oxygenation pattern) have both been explored for use against cancer in a number of studies.[155–165] Several studies have shown perillyl alcohol to have anticancer properties, such as chemopreventative activity against skin carcinogenesis and related skin damage,[165] as well as activity against lung cancer[158] and breast cancer cells.[157] It has also been the subject of several clinical trials.[166,167] Interestingly, the defense spray of *Parectatosoma mocquerysi* (predominantly containing parectadial (**12**)) has been observed to primarily affect the skin (Fig. 6). Thus, considering this property and by analogy to perillyl alcohol, parectadial may have cytotoxic or cytostatic properties which merit its investigation as an anticancer agent.

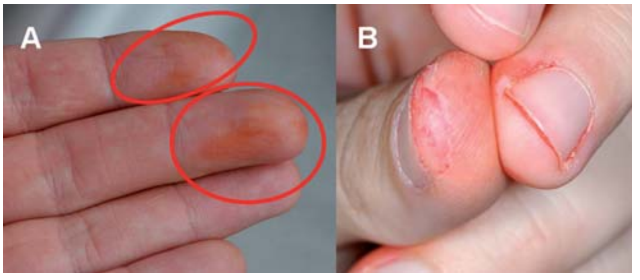


Fig. 6 Effects of defensive spray from the stick insect *Parectatosoma mocquerysi* on human skin.[132,138,143] Photo provided by Oskar V. Conle (Bolsterlang, Germany).

In addition to secondary metabolites, glucose (**25**) has recently been identified in the defensive spray of *Anisomorpha buprestoides*,[110,112,152] *Peruphasma schultei*,[110,112] *Parectatosoma mocquerysi*,[132] *Phyllium westwoodii*,[131] and *Megacrania nigrosulfurea*.[78] Boland *et al.* have published multiple reports of eloquent studies describing the role of glucose in sequestration, production, and transport of defensive compounds in the larvae of Chrysomelid beetles.[168–171] Glucose (**25**) may also be involved with pathways in the biosynthesis and/or transport of these substances into defense glands of phasmids.[110,112,131,132] As previously mentioned, phasmids have several important properties which make them attractive model organisms for biosynthesis studies. The identification of glucose and analogies to the defense chemistry of chrysomelid beetles also suggest that elucidation of similar pathways in phasmids might be easily achieved. Studies of chemical defense studies on phasmids are currently ongoing.

3.3 Order Hymenoptera (ants, bees, and wasps)

The order Hymenoptera contains over 115,000 species worldwide,[172] making it the second largest order of insects. It contains the largest group of organisms on earth which possess a chemical defense which is injected directly into the victim. Venoms in general are of particular interest to natural product drug discovery due to the obvious pharmacological effects they have on their targets. At least one comprehensive book has been published on the topic of Hymenoptera chemical defenses.[98]

In addition to compounds derived directly from the venoms or bodies of Hymenoptera, many important studies have been conducted on substances derived indirectly from these creatures. For example, the compounds isolated from the famous poison dart frogs are almost entirely derived from their arthropod diets, and a substantial portion come from ants. A number of decahydroquinoline derivatives were found in dendrobatid frogs as well as in extracts of the ant *Solenopsis azteca*.[42] Also, natural products from bees, such as their venom and materials like honey and propolis, have been used extensively for therapeutic purposes (see also Section 4.3).

A recent study of ant venoms illustrates nicely the potential that exists for new natural product discovery in species which have already been extensively studied previously. The red imported fire ant, *Solenopsis invicta*, was introduced into the USA (Mobile, AL or Pensacola, FL) from cargo ships coming from Brazil between 1933 and 1945, and has spread into at least 18 states and has been studied for several decades.[173] The primary active components of venom from this and related species have long been known to be a collection of piperidine alkaloids called solenopsins (**26–33**, Fig. 7).[174–177]

In 2009 Chen *et al.* identified 14 analogues of solenopsins from *S. invicta* (**34–48**), 11 of which were novel, utilizing new isolation techniques which they developed.[174] Given the fame and widespread range of this very invasive pest, the discovery of new venom compounds further illustrates the need to increase exploration of insects as sources of new natural products, even species whose chemistry has been studied in the past.

Besides the solenopsins from fire ants, other species of ants make a variety of alkaloids. A recent report by Jones *et al.* describes an example of ant-derived alkaloids from the ant

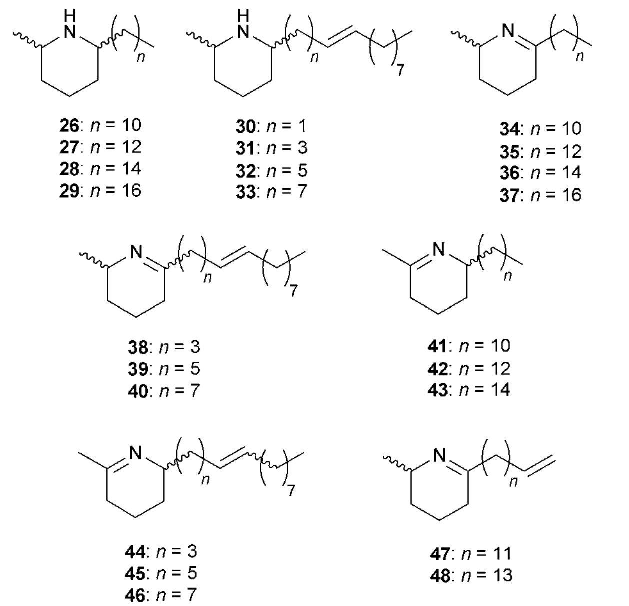


Fig. 7 Solenopsins, the major active substances from the venom of ants in the genus *Solenopsis* (such as *S. invicta*).[174–177,280] Where the relative configurations are ambiguous in this figure, either multiple stereoisomers were identified in the original report or no relative configuration was reported.

species *Myrmicaria melanogaster* of Brunei.[178] This single species of ant was found to contain 14 different alkaloids (**49–55**), whose core structures are illustrated in Fig. 8. In addition to alkaloids, ants have been shown to use other organic compounds for chemical defense, such as iridodial (**7**), dolichodial (**5**), and actinidine (**9**), among many others. However, only a tiny fraction of species have been analyzed to date.

4 Drug discovery potential of insects and their chemical defenses

Natural products are well established and proven sources of drugs and medicinally relevant substances. Over 70% of drugs on the market are, are derived from, or are based on natural compounds.[179] Compounds produced by Nature are well

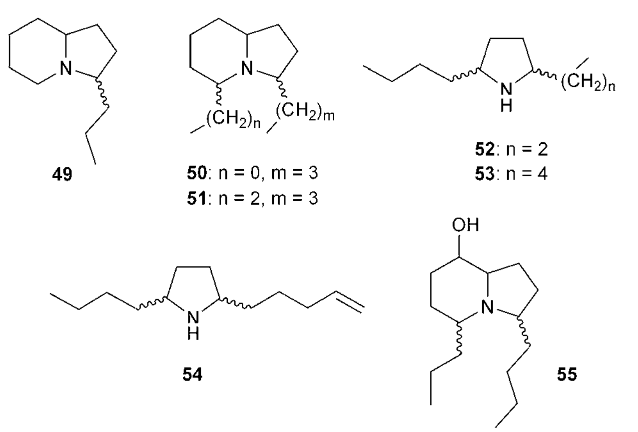


Fig. 8 Alkaloids from the Bornean ant *Myrmicaria melanogaster*.[178] Where the sidechain relative configurations are shown as ambiguous, either multiple stereoisomers were identified in the original report or no relative configuration was reported.

optimized to perform such functions as binding to specific target proteins[180] or interacting with membranes, among others. There are many properties which might make toxic substances (venoms, *etc.*) attractive as drug lead compounds including: 1) cytotoxicity, 2) neurotoxicity, and 3) efficacy against microbial pathogens. Insects produce thousands of different compounds with all of these properties, as well as other biological activities relevant to various disease states.

Medical entomology is most often focused on various aspects of arthropod disease vectors, such as insects (mosquitos, *etc.*) that carry disease. However, relatively little has been published about the vast potential that insect chemical biodiversity has to play in drug discovery and other endeavors of biomedical science, a field that has been given the name 'pharmaceutical entomology'.[45] This topic has been broached in only a few publications. For example, Metcalf has stated that insects have "become the 'new frontier' for natural products chemistry".[2,181] A recent review by Laurent *et al.* made only a brief mention of a few examples where defensive chemicals produced by insects have been pursued for practical medicinal application.[44] However, to date no comprehensive review or book chapter has been published on this topic. Ramos-Elorduy *et al.*[182] suggests that "insects … may prove a valuable source of prototype drugs".

It appears that only two large-scale surveys of arthropods have been conducted to date: one in the 1960s, which continues today in the group led by Pettit (described in Section 4.1);[183–185] and one by a group led by Trowell.[45] Pettit recently reiterated his earlier enthusiasm for the prospect of finding valuable natural products from arthropods. In a 1968 letter to the editor of the journal *Cancer Research* he stated that "From the information now at hand, the arthropods appear a new and interesting source of potentially useful antineoplastic agents."[184] Recently, he stated that "My expectations for the discovery and development of new anticancer constituents contained in certain arthropods has not changed [since that article] but instead has definitely intensified." (G. R. Pettit, personal correspondence, 20th July 2010). Arthropod-based drug discovery by the Trowell group began in 2002 at Australia's Commonwealth Scientific and Industrial Research Organisation (CSIRO) and spun off into the company Entocosm. Those studies began with over 1000 arthropod species, of which 80% were insects, that included representatives from 17 different orders of insects.[45] Both Entocosm[45] and Entomed[186] were founded with the mission of discovering drugs in arthropods. Costa-Neto cites one example in which the pharmaceutical company Merck has patented a scorpion toxin called margatoxin for use as an immunosuppressant.[18] In 1991, the National Biodiversity Institute of Costa Rica (INBio) entered into a US$1 million agreement with Merck aimed at helping Costa Rica develop resources to protect its biodiversity effectively and to test insect extracts for their efficacy against infections, AIDS, cancer and inflammatory conditions.[187,188] Nonetheless, very few, if any, labs at academic or government institutions predominantly focus on insects as potential sources of drugs. New advances in analytical chemistry, new disease models for more efficient and high-throughput drug screening, as well as epidemics of emerging pathogens and other diseases, provide significant rationale for deeper exploration of arthropods as sources of new drugs.

A number of articles describing specific examples of insect-derived substances with medically relevant properties have been published. For example, insect-derived peptides represent a vast and relatively unexplored resource for drug discovery. In fact, the vast majority of anticancer, antimicrobial, and antiviral substances isolated from insects and subsequently studied have been peptides.[189,190] Thus, even bioinformatic, proteomic, or genomic methods in lieu of traditional isolation and characterization may prove fruitful in searching insects for antibiotics. However, the rather small sequence homology among insect antimicrobial peptides as a whole makes targeted searches for peptides with specific biological activities difficult, regardless of peptide family.[190,191] Additionally, in a very recent study on proteins from the venom of the ectoparasitic wasp *Nasonia vitripennis*, de Graaf *et al.* reported finding 79 different proteins in that species' venom, half of which had not been previously associated with insect venoms.[192] Those researchers used a combination of bioinformatics and proteomics (mass spectrometry), with very few proteins identified by both techniques (see Fig. 2 from that article).[192] This appears to further illustrate that, while genomics and bioinformatics are powerful methods, many interesting and useful natural products, even proteinaceous ones, might be missed without complementing those approaches with chemical/isolation methods. This work also demonstrates that a huge number of new proteins with high potential for pharmacological activity exist in the many thousands of unexplored insect species. Additionally, only a few insect species have had their genomes sequenced, and only a tiny fraction of insect species have any of their genetic or protein sequences published. Thus, the data infrastructure doesn't yet exist for purely bioinformatic approaches to biologically active protein/peptide identification and bioprospecting in insects. Finally, most bioinformatic methods will not lead to identification of non-peptide small-molecule secondary metabolites, given our lack of knowledge about the vast majority of small-molecule biosynthetic pathways in insects.

Insects are well known for producing a huge variety of chemical substances with potent biological activities for a host of ecological functions such as communication and defense.[44,59,86,87,89,91,110,112,131] Chitin itself, the major component of arthropod exoskeletons, and at least one of its derivatives, chitosan, have even been shown to possess multiple physical and chemical properties useful for a variety of applications relevant to medicine, such as wound healing and reducing cholesterol.[2,17,79,80,193] Ramos-Elorduy *et al.*[194] and Andray *et al.*[195] used chemical screening to confirm the presence of proteins, terpenoids, sugars, polyols, saponins, polyphenolic glycosides, quinones, anthraquinone glycosides, cyanogenic glycosides, and alkaloids in just 14 species of insects.[2,17] In addition to the therapeutic potential for arthropod neurotoxins, Konno *et al.* and others have stated that many of these substances also hold great potential for basic neuroscience and other fundamental biological research areas.[18,196] Usefulness in probing basic science and fundamental biological questions represents yet another vast layer of the potential held by arthropod natural products in general. Given the sheer numbers and diversity of insect species on the planet, improved and continued use of modern analytical techniques in traditional bioassay-driven isolation, identification, and synthesis approaches to finding new pharmaceuticals in insects is still a very promising endeavor. The following examples make it even clearer that insects are potentially valuable, yet largely underexplored, resources for natural product drug discovery and bioprospecting in general.

4.1 Cytotoxins and anticancer compounds

Venoms and other toxic substances produced by various organisms are very attractive candidates for drug development.[197] By virtue of their toxicity or ecological role in paralyzing prey, warding off predators, or both, they often have a known physiological effect on animals such as humans. Cytotoxicity (cell-killing) substances are often pursued as anticancer chemotherapeutics.[198] This is a prime example of how chemical ecology can and does inform pursuits in pharmacognosy and drug discovery on which substances may be most promising to study. For example, the cytotoxic amine farnesylamine, an inhibitor of farnesyl protein transferase, was studied for its pharmacological properties even before it was identified as a natural product (its first verified natural source being the Australian ant species *Monomorium fieldi*[199]). Additionally, Oldfield[200,201] reported that in the 1970s, over 4% of the extracts from over 800 terrestrial arthropod species (insects, spiders, crustaceans, millipedes, and centipedes) evaluated for anticancer activity were found to have some amount of activity.[2,17] This work, along with the studies by Trowell *et al.*,[45] appear to be the only large-scale survey of insects as a source of potential cancer drugs to date.

One example of a cytotoxin from insects being explored for potential medical application is cantharidin (**1**) from blister beetles (family Meloidae). Even though the previously mentioned traditional uses to enhance human sexuality are likely without merit (see Section 2.3), cantharidin has other properties which have been explored for use in treating other diseases in which it may have some efficacy. The blistering of the skin caused by cantharidin is due to the death of skin cells, which suggests potential efficacy against cancer.[22] Indeed, cantharidin and its chemical derivatives have been explored due to its cytotoxic and apparent anticancer properties.[22,202,203] Additionally, due to its cytotoxic effects on skin, it is commonly used even in modern medicine for wart removal.[22]

Even some common fatty acids from insects have been shown to possess anticancer properties. Yoo *et al.* reported that several such compounds were isolated from the flower beetle *Protaetia brevitarsis*.[49] In that study, the scarab larvae (or grubs) were extracted with dichloromethane and that extract was further fractionated on silica gel. One fraction which possessed anticancer activity was further analyzed by both NMR and GC–MS. That fraction was found to contain predominantly two fatty acids: palmitic acid and oleic acid. Yoo *et al.* also reported that an authentic standard of palmitic acid induced apoptosis in colon cancer cells. It was shown previously that high concentrations of some fatty acids cause cell death by apoptosis or necrosis,[204] and that palmitic acid can induce apoptosis in some cancer cell lines.[205]

The solenopsins **26–48** (mentioned in Section 3.3) from red imported fire ants (*Solenopsis invicta*) and related species have been pursued for a variety of medicinally relevant applications due to their ability to elicit necrosis in human tissue.[176,177,206] They have been investigated for their ability to inhibit angiogenesis,[207] as inhibitors of nitric oxide production,[208] as well as for their effect on the nervous system and on cardiosuppression in humans.[209]

In addition to venoms, other substances from insects can prove to be potent toxins that may be useful for fighting cancer. For example, in the 1960s, the research group led by Pettit began the first large-scale survey of arthropods for medicinal compounds.[2,17,183–185,200,201,210–214] These studies have continued to produce discoveries even as recently as this year, including the following examples.[185] This work has resulted in identification of anticancer activity from insects such as the Asian rhino beetle (*Trypoxylus* (*Allomyrina*) *dichotoma*), the Asian butterfly *Catopsilia crocale*,[215] and the wasp *Vespula pennsylvanica*.[200,201,210,213] More recent examples include the Texas lubber grasshopper *Brachystola magna*[210] and the Asian butterfly *Byasa polyeuctes termessa*.[185] In 2005 three antineoplastic agents (**56–58**, Fig. 9) were isolated from grasshopper specimens (*Brachystola magna*) which had been collected in Texas in 1967 and preserved in isopropanol.[210] Isoquinoline derivatives were previously only known from amaryllidaceous plants, which are not among this grasshopper's usual diet. However, the authors of that work postulate that those compounds may have come from other plants on which the grasshoppers feed, and that they might sequester them as part of a defensive strategy. Nonetheless, it is fascinating to think that even an old jar of grasshoppers could potentially hold the cure for cancer. More recently, Pettit *et al.* published identification of new cytotoxic substances from butterfly extracts which were a part of their earlier arthropod anticancer survey work.[185] In this study, ethanolic extracts of the butterfly *Byasa polyeuctes termessa* from 1967 were subjected to activity-guided fractionation and tested for activity against the mouse-derived leukemia model P388. This resulted in the isolation of a new cancer cell growth inhibitor, papilistatin (**59**).[185] Pettit *et al.* point out that papilistatin is very similar in structure to aristolochic acid, present in other Asian butterflies that feed on foodplants in the same genus (*Aristolochia*) as do *B. polyeuctes termessa*.

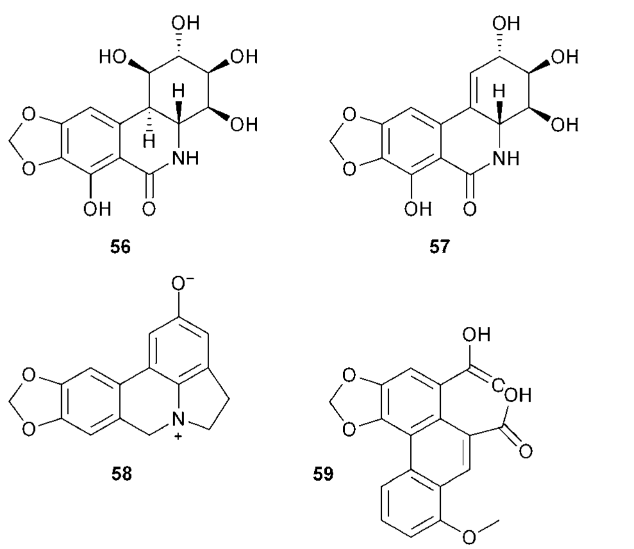


Fig. 9 Cytotoxic compounds from the Texas lubber grasshopper *Brachystola magna* and the butterfly *Byasa polyeuctes termessa*.

At least one intriguing example of an insect-derived antitumor compound comes from the order Diptera (flies). The peptide derivative *N*-β-alanyl-5-*S*-glutathionyl-3,4-dihydroxyphenylalanine (5-*S*-GAD, **60**, Fig. 10) was first discovered as an antibacterial substance from immunized larvae of the flesh fly species *Sarcophaga preegrina*[216] (see Section 4.3 for a discussion of its antibacterial properties). It has even been shown very recently to delay the progression of UV-B-induced cataract when administered to the eyes of UV-B-exposed rats.[217] The same group tested 5-*S*-GAD on human cancer cells, with potencies in the range 0.5–20 μM on 38 cancer cell lines such as melanoma and breast carcinoma.[159,218] The compound had previously been shown to induce production of H_2O_2[216] (known to be cytotoxic as well as antibacterial), and H_2O_2 was found in the culture medium of cells treated with 5-*S*-GAD. For the breast cancer cell line MDA-MB-435S, both 100 μM 5-*S*-GAD and 100 μM H_2O_2 performed equally well in time-course cytotoxicity experiments. However, the other breast cancer cell line tested, T47D, responded only to H_2O_2, suggesting a different level of sensitivity to H_2O_2. T47D cell culture medium also did not accumulate H_2O_2 to the same extent as did that of MDA-MB-435S cells upon the addition of 5-*S*-GAD. Addition of catalase to cell culture media abolished the cytotoxicity in a dose-dependent manner in both cell lines tested. Additionally, it was observed that addition of superoxide dismutase (SOD) to the cell culture medium also abolished cytotoxic activity of 5-*S*-GAD. Thus, this report demonstrated that the cytotoxic activity of 5-*S*-GAD is, at least in part, due to its ability to facilitate accumulation of H_2O_2 and O_2^- *in vitro*.[218]

Often insects efficiently concentrate useful substances from their diet or other features of their environment. For example, the nests of wasps have been shown to contain anticancer substances. Many so-called 'paper wasps' (family Vespidae) make their nests out of cellulosic plant material collected from a variety of sources. In the first report of an anticancer quinine from wasp nests, Fujiwara *et al.* described the isolation of 7,8-seco-*para*-ferruginone (**61**) from nests of the social wasp *Vespa simillima*.[219] This compound, and its possible precursor ferruginol (**62**), also occurs in the bark of at least one plant that this wasp collects nest building materials from, Japanese cedar (*Cryptomeria japonica*) (Fig. 11). However, **61** is present in much higher concentrations in the wasp nests than it is in the bark of that tree. Thus, the authors of that report logically postulate that this wasp may preferentially collect bark from *C. japonica* and, as a result, use ferruginol (**62**) as a precursor to generate **61**. In

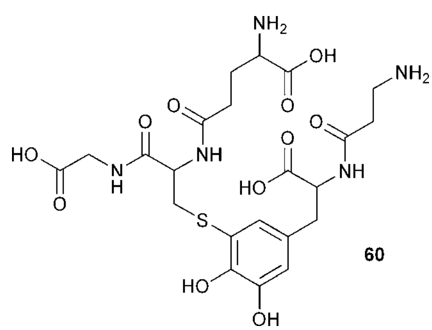
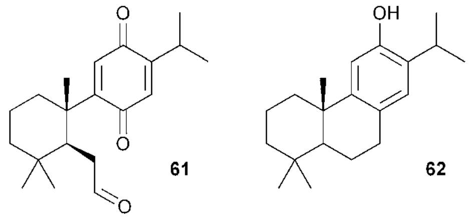


Fig. 10 5-*S*-GAD (**60**) from the flesh fly *Sarcophaga peregrina*.[159,216–218]



61 62

Fig. 11 Compounds from the nests of *Vespa simillima* (**61**) and from a likely source of its nest material, *Cryptomeria japonica* (**62**).[219]

either case, the insects are actively concentrating the quinine in their nest, thus making the nests of these insects a more likely natural matrix in which to find such substances.

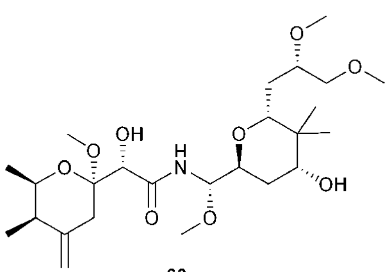
As a final example of a potential anticancer compound from insects, the polyketide derivative pederin (**63**, Fig. 12) is a compound discovered in the hemolymph of *Paderus fuscipes* (family Staphylinidae).[220] It was discovered in 1953 by Pavan and Bo from the extract of over 25 million field-collected beetles.[220,221] The hemolymph of *Paderus* beetles has long been known to cause blistering and inflammation on human skin. Pederin is the primary component responsible for this property. It is produced by an internal symbiont of the beetle *Pseudomonas aeruginosa*, and is believed to function in the beetles as a chemical defense, being effective against spiders.[44,221] Pederin's toxic properties make it an attractive candidate in anticancer studies. Multiple analogues of pederin have been shown to have significant cytotoxicity, and there are at least 36 members of the pederin family, from both terrestrial and marine sources, that have been characterized so far.[221]

4.2 Neurotoxins

Within the phylum Arthropoda, spider venoms are the most famous arthropod toxins that have been studied, for a variety of reasons. Spiders, though not as numerous as insects, are a large and diverse group of animals, with about 40,000 species grouped into 110 families which contain 3618 genera.[222] Most spider venoms contain neurotoxins which are used to paralyze prey rather than for defense. Estrada *et al.* recently published a very thorough review on acylpolyamines and peptides from a variety of spiders and their potential use in treating pain and central nervous system (CNS) diseases such as Huntington's, Alzheimer's and Parkinson's diseases.[222] Another paper by Schroeder *et al.* utilized modern NMR technology and mixture analysis to identify novel sulfated nucleosides in the venoms of over 70 different species.[223] Some of these species, such as the brown recluse (or violin spider, *Loxosceles reclusa*) and hobo spider (*Tegenaria agrestis*) are common pests, and have been well-known for their toxicity for many decades. In addition to the nucleotides, the study by Schroeder *et al.* also identified a polyamine compound very similar to a well known wasp venom compound called philanthotoxin[224–226] (see below). In fact, over 40 different polyamine neurotoxins have been isolated from the venoms of spider species.[222,225] Thus, even though spiders are an established source of toxins, they still represent a vast reservoir for discovery of useful substances such as potential pharmaceuticals. Since spider and scorpion (class Arachnida) venoms are well-studied and well-reviewed, this article will not discuss further examples from this group.

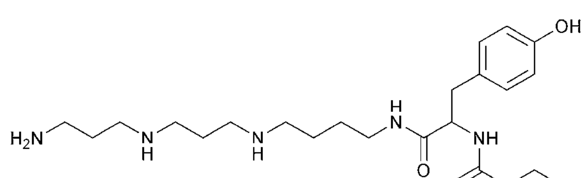
The venoms of many insects, particularly those of ants, bees, and wasps (order Hymenoptera) contain neurotoxins. For example, philanthotoxin (**64**, Fig. 13) was originally discovered in the venom of the predatory wasp species *Philanthus triangulum*.[224–226] The predatory nature of this wasp, along with the aforementioned similarity in structure of a compound found in spider venom with that of philanthotoxin, suggests a possible convergent evolutionary relationship between these two neurotoxins, both presumably used to subdue prey. Philanthotoxin is a noncompetitive antagonist of both glutamate and nicotinic acetylcholine receptors.[225] These receptors in humans have a wide variety of functions, including pre- and post-synaptic neural transmission, memory formation, learning, and muscle contraction. They are, therefore, major drug targets for a number of therapeutic applications, including treatment of neurodegenerative diseases. Several structure–activity relationship (SAR) studies have used a medicinal chemistry approach of producing over 100 analogs of philanthotoxin to improve specificity, using photolabile analogs to probe receptor structure[225] and to design more selective antagonists of specific human receptors, with the goal of generating more useful compounds with therapeutic potential.[224,227] Work on philanthotoxin, as well as many other invertebrate-derived neurotoxins, has also demonstrated that such insect-derived toxins are valuable in basic neuroscience research as well as for the discovery of potentially useful drugs.[227]

Another example of a potent hymenopteran neurotoxin, the venom of the bullet ant (*Paraponera clavata*) is reported to be the most painful ant venom in the world. This venom contains a protein called poneratoxin (previously discussed in Section 2.1). This protein blocks voltage-dependent ion channels in insects. It has even been proposed as a potential insecticide if expressed by an insect virus.[228] There have been no medicinal applications for poneratoxin or its homologs from other ant species proposed to date, but its striking effect on humans



63

Fig. 12 Pederin (**63**), a defensive compound from the rove beetle *Paederus fuscipes*.[44,220,221]



64

Fig. 13 Philanthotoxin (**64**), a neurotoxin from venom of the predatory digger wasp *Philanthus triangulum*.[224–227] strongly suggests its potential to contribute to the development of new drugs.[229]

4.3 Antibiotics

With the appearance of newly emerging pathogens and various antibiotic-resistant microbial diseases, the search for new antibiotics is a particularly important goal of modern drug discovery. Insects, as for all other organisms, are susceptible to infection by microorganisms. Various antimicrobial substances have been found in insects,[56,57,83,84,189–191,216,230–251] many of which likely function as a defense against microbial attack and infection. It has been shown that in the various steps a microbe must take in order to infect an insect, from the cuticle to the hemocele (body cavity), it encounters a large and varied assortment of antimicrobial substances such as lipids, hydrocarbons, diphenols, carbohydrates, melanin, and even the insect's own chitin exoskeleton.[2] One major example of such substances are insect antimicrobial peptides.[189–191,233–238,242,243,247–251] In fact, cecropins were the first antimicrobial peptides discovered.[233–235,252] Defensins (Table 1), another common class of insect antimicrobial peptides, are found in a wide variety of organisms from plants to insects to humans, and their biological activity against bacterial and fungal pathogens has been reported.[253] Melittin (a major component in the sting venom of honey bees such as the European honeybee, *Apis mellifera*; order Hymenoptera) and cecropins are both effective against Gram-negative bacteria.[251] Genes encoding insect antimicrobial peptides have even been pursued for their potential use in transgenic plant production for agricultural applications.[254]

Antimicrobial substances are important tools in the innate immune system for many animals such as insects. Innate immunity is the first line of defense against infection for most animals, as opposed to the slower and more specific adaptive immune system. Most of the known antimicrobial substances produced by insects are peptides, but in some cases antimicrobial small-molecule secondary metabolites have been found.[216,230,232,240,241,245,246,255] Often, chemical defenses against microbial infections are found in the hemolymph (blood) of the insects rather than in a spray or secretion. For example, as mentioned above, cecropins were the first antimicrobial peptides ever discovered in animals.[233–235,252] They were originally isolated from the pupae of (and thus named after) cecropia moths (*Hyalophora cecropia*; order Lepidoptera). At least two families of antibiotic peptides have been found in cecropia moths, including the cecropins and the attacins.[189–191,233–235,237,238,242,243,247–251] Cecropins and defensins have subsequently been found in a number of other insect species[238] as well as other organisms.[248] Additionally, a large body of work on innate immunity in insects has been the focus of the laboratory of Hoffmann.[239] A more recent discovery of the long-sought antimicrobial factor from the above-mentioned medicinal maggot species *Lucilia sericata*, a species used clinically for better and faster wound healing, was named lucifensin (Table 1) and published by Čeřovský *et al.* in 2009.[236] This peptide was found in various tissues of the insect as well as its secretions/excretions, and found to be effective against *Micrococcus luteus*. Its sequence was determined by mass spectrometry and found to be analogous to defensins. Indeed insect-derived antimicrobial peptides represent a vast reservoir of very promising biologically active compounds. However, since antimicrobial peptides have been extensively reviewed in a number of recent publications cited above, I will not discuss this topic further here.

Flies (Order Diptera) may also be a valuable resource for antimicrobial agents. Fly larvae (maggots) have been used for centuries to aid wound healing by quickly removing dead tissue while simultaneously protecting against infection.[31,52] Antimicrobial substances have been isolated from fly larvae in several recent studies.[31,232,240,241,244,246,247] In those publications, the variety of compound types with antimicrobial activity ranges from small organic metabolites such as lipids[246] and others[241] to antibacterial proteins[247] and even some unidentified compounds.[240,244] In fact, several substances isolated from larvae of *Lucilia sericata*, in addition to the previously mentioned lucifensin, have been shown to be effective against a range of bacterial pathogens including MRSA.[232,240,244] It has also been shown that a variety of insect genes, including immune defense genes and genes involved with antimicrobial substance production, have increased expression when challenged with *Escherichia coli* endotoxin.[256] Most of these studies have looked at only one species. However, many other flesh-eating fly species exist, and others are also used for wound healing. In fact, the microbial complexity that exists in such environments as a rotting carcass is far greater than that of a treated human wound. Thus, flesh-eating flies represent a vast potential reservoir of antibiotics for treating microbial infections. In another example from the order Diptera, an antimicrobial lipid, 1-lysophosphatidylethanolamine (C16:1) (**65**) (Fig. 14), has been isolated from the common house fly (*Musca domestica*). This compound was shown to inhibit growth of the Gram-positive bacterium *Bacillus thuringiensis* and the yeast *Saccharomyces cerevisiae*, but not the Gram-negative bacterium *Escherichia coli*.[246]

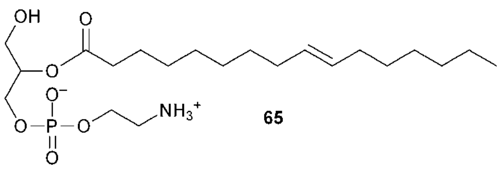
Table 1 Antimicrobial and antiviral peptides isolated from insects

Peptide	Sequence	Species	Antimicrobial activity	Antiviral activity
Attacin[242,277]	(188 amino acids)	*Hyalophora cecropia*	✓	
Alloferon 1[237]	HGVSGHGQHGVHG	*Calliphora vicina*	✓	✓
Cecropin A[252]	KWKLFKKIEKVGQNIRDGIIKAGPA VAVVGQATQIAK	*Hyalophora cecropia*	✓	✓
Defensin A[238,278]	ATCDLLSGTGINHSACAAHCLLRGNRGG YCNGKGVCVCRN	*Phormia terranovae*	✓	
Lucifensin[236]	ATCDLLSGTGVKHSACAAHCLLRGNRGG YCNGRAICVCRN	*Lucilia sericata*	✓	
Melittin[189,279]	GIGAVLKVLTTGLPALISWIKRKRQQ	*Apis mellifera*	✓	✓

Another example of a fly-derived insect antimicrobial substance is 5-*S*-GAD (**60**), whose cytotoxic properties were mentioned in Section 4.1. In fact, the antibacterial properties of 5-*S*-GAD as a likely immune anti-infective defense substance in flies are more consistent with biorational logic than are its antitumor effects. Likewise, it was first characterized as an antimicrobial substance effective against both Gram-positive (*Micrococcus luteus*) and Gram-negative (*Escherichia coli* and *Staphylococcus aureus*) bacteria.[216] It was also demonstrated that 5-*S*-GAD was associated with H_2O_2 production, which was proposed to be a novel antibacterial mechanism. The authors also proposed that 5-*S*-GAD protects the fly larvae through H_2O_2 production, which up-regulates *Rel* family transcription factors such as NF-κB, which results in the expression of additional antibacterial proteins in the insects. Interestingly, NF-κB is implicated in a variety of anti-infective and anti-stress pathways[257] which are briefly discussed in Section 4.4.

In a report by Huberman *et al.*, several antimicrobial substances were isolated from the previously mentioned blowfly *Lucilia sericata*.[241] This was a significant study in the field in several ways. In the report, three compounds were identified from larval and hemolymph extracts of the fly using GC–MS and antibacterial assays: *p*-hydroxybenzoic acid, *p*-hydroxyphenylacetic acid, and octahydrodipyrolo[1,2-*a*;1′,2′-*d*]pyrazine-5,10-dione (the cyclic dimer of the amino acid proline). This study was the first to isolate the cyclic dimer of proline from insects, and demonstrate its antibacterial activity. These researchers extracted *L. sericata* larvae and hemolymph and used zone-of-inhibition assays, colony-forming unit (CFU) counts, and liquid culture turbidity tests to screen fractions for antibacterial activity against *Micrococcus luteus* and *Pseudomonas aeruginosa*. The compounds *p*-hydroxybenzoic acid and *p*-hydroxyphenylacetic acid are both used as preservatives in the food and cosmetic industries.[258] Additionally, *p*-hydroxybenzoic acid is produced by aquatic beetles, presumably to prevent microbe attachment to beetles, which would disrupt their ability to repel water.[259] A similar compound, *p*-hydroxycinnamaldehyde, is an antimicrobial isolated from another Dipteran, the sawfly *Acantholyda parki*.[245] In addition to this cyclic proline dimer, other dipeptides isolated from flesh flies (*Sarcophaga peregrina*[216] and *Neobellieria bullata*[230]) have been shown to possess antimicrobial properties.[241]

In addition to Lepidoptera and Diptera, antimicrobial properties have been discovered in substances derived from other orders of insects such as Coleoptera[260] and Hymenoptera. For example, several studies have verified antimicrobial activity in the products of bees such as honeybee propolis. Although there are many examples, the topic of biological activity and identification of biologically active compounds in bee propolis has been extensively reviewed.[55,57,58,83,84,182] Thus, this review will not further discuss these properties of propolis, and refers its readers to those publications. Glandular secretions present in the nests of ants have also been shown to possess antifungal properties.[231] Other social insects, such as social bees, also use antimicrobial substances to construct their nests.[261] It has also been postulated, with examples cited, that soil-dwelling insects may also have a higher incidence of antibiotic production due to the microbial complexity of their immediate environment.[26] The production of antimicrobial substances by social and soil-dwelling insects makes a lot of sense, since they presumably come into contact with common sources of pathogens more frequently than other insects.



65

Fig. 14 1-Lysophosphatidylethanolamine (C16:1) (**65**), an antimicrobial lysophospholipid from the housefly *Musca domestica*.[246]

4.4 Antivirals

Some insect substances, particularly peptides (Table 1), have also been shown to be effective against virus infection and replication. In the aforementioned review by Slocinska *et al.*, examples of insect-derived peptides with antiviral activities were cited.[189] For example, anti-HIV activity has been reported for both melittin and cecropins.[250,262–264] Melittin (from the sting venom of honeybees) and its analogs have also been shown to be effective against other viruses such as herpes simplex virus[191,265] and Junin virus.[191] Several of these peptides, such as melittin and their analogs may function similarly in their antimicrobial and antiviral activities.[189] Melittin and cecropins are both effective against Gram-negative bacteria.[251] Melittin causes lysis of membranes of both Gram-negative bacteria[251] as well as the membranes of enveloped viruses such as murine retroviruses.[266] Alternatively, melittin has anti-HIV activity well below virolytic levels,[263] and also functions to inhibit HIV gene expression.[250] Another group of insect-derived antiviral peptides, originally discovered in the hemolymph of experimentally infected blowflies (*Calliphora vicina*), are the alloferons.[237] These peptides have been shown to be effective against influenza and herpes simplex virus.[189,257] A detailed study found that one possible mode of action for alloferons against viruses could be activation of the NF-κB signaling pathway,[257] which is found in nearly all animal cell types and is involved with cell stress, free radicals, and antigens from bacteria and viruses, among other things. Its disruption can lead to viral or bacterial infection (due to reduced immune response), inflammatory disease, and cancer.[267,268] In fact, alloferons also have anticancer properties.[237]

4.5 Other medicinally relevant properties

Some insect-derived substances have been shown to have biological activities related to a host of other diseases besides infections and cancers through mechanisms besides cytotoxicity. For example, bee venom therapy is commonly used to treat a variety of conditions such as arthritis, rheumatism, pain, and even cancer. It contains a variety of proteins and other substances with multiple pharmacologically relevant properties.[53] The use of bee venom and other natural products from bees is known as apitherapy. There is even an organization dedicated to promoting apitherapy, the American Apitherapy Society. As far back as about 2500 years ago, Hippocrates reportedly prescribed bee stings for therapeutic purposes.[2,82] In addition to the antimicrobial, anticancer, and antiviral activities mentioned above, mellitin and other components of bee-sting venom have been shown by many labs to have biological activities relevant to those diseases and possibly others.[53] DNA coding for melittin along with gene therapy methods have even been shown to have effects against certain cancers both *in vivo* and *in vitro*.[269,270]

Powdered silkworm larvae (silkworm powder) are often prescribed in Asian medicine and are commercially available. Silkworm powder has been tested and shown in modern bioassays to inhibit absorption of glucose in human intestinal epithelium cells[20] and reduce vasopressin expression in the hypothalamus of diabetic mice.[271] The components responsible for the anti-diabetic activity of silkworm powder are likely to include sugar-mimetic α-glucosidase-inhibiting alkaloids such as 1-deoxynojirimycin (DNJ)[20,272] (**66**) and other sugar-mimetic alkaloids including 1,4-dideoxy-1,4-imino-D-arabinitol (D-AB1) (**67**), and 1,4-dideoxy-1,4-imino-D-ribitol (**68**, Fig. 15).[272]

These compounds have been shown to be sequestered by caterpillars of *Bombyx mori* from their diet, mulberry (*Morus* sp.) to nearly 3 times the concentration of mulberry leaves using specialized enzymes.[20] Caterpillars of other species fed on mulberry did not survive longer than 4 days, suggesting these alkaloids have a defensive function for the mulberry trees against herbivory against Lepidopteran pests.[272] It is also possible that sequestration of these alkaloids once served a defensive function for *B. mori* itself, but wild populations of this species no longer exist and studies testing this hypothesis have not yet been published. Nonetheless, this is yet another example of defensive toxins with potential medicinal value.

5 Natural products from arthropod-associated microbes

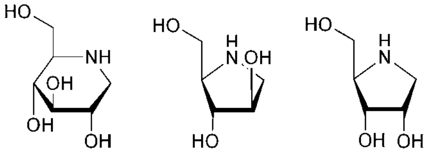
Natural products from fungal and microbial sources,[179] including insect-associated microbes, fungi, and symbionts,[10] have gained much attention in the field of natural products chemistry in recent years as rich sources of compounds and very useful models for studying their biosynthesis. Studies of biosynthetic pathways can be useful for mass-production of known compounds of value, as well as generation of libraries of new compounds with potentially new or improved uses. Indeed, a chemical ecology approach to isolating the most promising sources of compounds from microbes, including considerations of ecological function and their roles in symbiotic interactions with multicellular organisms such as insects, will likely contribute to discovery of new medicinally relevant compounds in coming years. However, microbial organisms are beyond the scope of this review, and so only brief mention is warranted. One example involves the larvae of the beetle *Dendroctonus frontalis*, who depend for food on two species of fungi (*Entomocorticum* sp. and *Ceratocystiopsis ranaculosus*) in the wood of pine trees. An additional fungus, *Ophiostoma minus*, protects the beetles against the tree's chemical defenses. However, *O. minus* inhibits the growth of *Entomocorticum* sp.[10,273,274] Adding to the level of complexity in this system, an additional bacterial species produces a novel antibiotic compound called mycangimycin (**69**, Fig. 16) which inhibits the growth of *O. minus*, thus protecting the beetle's fungal food source.

Another promising example includes two novel cyclopeptides called hirsutatins that have been discovered from the entomopathogenic (insect-infecting) fungus *Hirsutella nivea*. Those peptides were shown to inhibit the human pathogen *Plasmodium falciparum* and the causative agent of tuberculosis, *Mycobacterium tuberculosis*.[275] Also from the world of insect-pathogenic fungi, a chlorinated compound, isariotin F, also showed significant activity against *P. falciparum* and *M. tuberculosis*, as well as the fungal pathogen *Candida albicans*. It also showed moderate cytotoxic activity against three cancer cell lines.[276]

6 Summary and conclusions

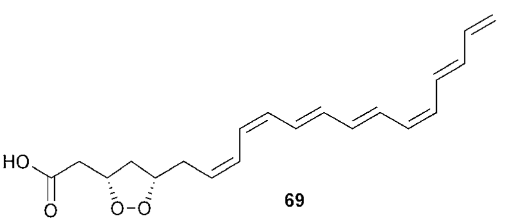
Insects make up the largest and most biodiverse group of organisms on the planet. Likewise, the magnitude of the chemical diversity which they produce and utilize is also one of the most impressive in the living world. Among the many uses of chemistry that insects employ to adapt and survive, their chemical defense mechanisms exemplify the chemical biodiversity that exists in this vast, varied and complex class of animals.

As modern drug discovery efforts move forward, natural products will continue to play a vital role in supplying medicine with chemical compounds that would otherwise be impossible for the human chemist to fathom. With modern technologies to analyze and assay ever-smaller amounts of material, it is important that previously neglected taxa and natural matrices are capitalized upon. Clearly, among these are insects and other arthropods, which possess one of the richest and most unexplored reservoirs of potentially useful substances. From toxins used to defend against attack by predators and other offending opponents to peptides which help to ward off infection by various microbes and other parasites, insects and their defense chemicals hold great promise for the future of natural products drug discovery.



66 67 68

Fig. 15 Sugar-mimic alkaloids from the mulberry *Morus australis*.[272]



69

Fig. 16 Mycangimycin (**69**) from the bacterial species *Streptomyces* sp. SPB74, a mutually beneficial symbiont of the southern pine beetle *Dendroctonus frontalis*.[274]

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8 References

1 A. J. Beattie, *Bioscience*, 1992, **42**(4), 290–292.
2 E. M. Costa-Neto, *J. Ethnobiol.*, 2005, **25**(1), 93–114.
3 L. H. Caporale, *Proc. Natl. Acad. Sci. U. S. A.*, 1995, **92**(1), 75–82.
4 T. Eisner, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**(Suppl. 2), 14517–14518.
5 T. Eisner and M. Berenbaum, *Science*, 2002, **295**(5562), 1973.
6 T. Eisner and J. Meinwald, *Chemical Ecology: The chemistry of biotic interaction*, National Academy Press: Washington, D.C., 1995.
7 J. J. Hellmann and N. J. Sanders, 'The extent and future of global insect diversity', in *Biodiversity Under Threat*, ed. R. E. Hester and R. M. Harrison, Royal Society of Chemistry: Cambridge, 2007, pp. 33–50.
8 J. Meinwald, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**(Suppl. 2), 14514–14516.
9 J. Meinwald, *Helv. Chim. Acta*, 2003, **86**, 3633–3637.
10 M. R. Berenbaum and T. Eisner, *Science*, 2008, **322**(5898), 52–53.
11 R. M. May, 'The dimensions of life on earth', in *Nature and human society: The quest for a sustainable world*, ed. P. H. Raven and T. Williams, National Academy Press: Washington, D.C., 2000, pp. 30–45.
12 A. Butenandt, R. Beckmann and E. Hecker, *Hoppe Seyler's Z. Physiol. Chem.*, 1961, **324**, 71–83.
13 A. M. El-Sayed, *Defense Substances*, http://www.pherobase.com/database/compound/compounds-behav-defces.php and http://www.pherobase.com/ (accessed 23rd July 2010).
14 E. D. Morgan, *Biosynthesis in insects*, Royal Society of Chemistry: Cambridge, 2004.
15 R. Bulmer, *Am Anthropol*, 1965, **67**(6), 1564–1566.
16 C.-X. Zhang, X.-D. Tang and J.-A. Cheng, *Entomol. Res.*, 2008, **38**(Suppl. 1), S38–S47.
17 E. M. Costa-Neto, *Hum. Ecol.*, 2002, **30**(2), 245–263.
18 E. M. Costa-Neto, *An. Acad. Bras. Cienc.*, 2005, **77**(1), 33–43.
19 G. R. DeFoliart, *Annu. Rev. Entomol.*, 1999, **44**, 21–50.
20 J. Han, S. Inoue and H. Isoda, *J. Nat. Med.*, 2007, **61**(4), 387–390.
21 P. James and I. J. Thorpe, *Ancient inventions* (1st edn), Ballantine Books: New York, 1994.
22 L. Moed, T. A. Shwayder and M. W. Chang, *Arch. Dermatol.*, 2001, **137**(10), 1357–1360.
23 T. Namba, Y. H. Ma and K. Inagaki, *J. Ethnopharmacol.*, 1988, **24**(2–3), 247–285.
24 K. Nonaka, *African Study Monographs*, 1996, **22**, 29–46.
25 P. Oudhia, 'Traditional knowledge about medicinal insects and mites in Chhattisgarh, India: An overview', conference paper at *Promotion and Development of Botanicals with International Coordination: Exploring quality, safety, efficacy and regulations*, Kolkata, India, 2005.
26 R. W. Pemberton, *J. Ethnopharmacol.*, 1999, **65**(3), 207–216.
27 J. Ramos-Elorduy de Conconi and J. M. P. Moreno, *J. Ethnobiol.*, 1988, **8**(2), 195–202.
28 B. C. Ratcliffe, *Coleopts Bull.*, 2006, **60**(5), 85–101.
29 P. Sandroni, *Clin. Auton. Res.*, 2001, **11**(5), 303–307.
30 T. H. Slone, *The Food Insects Newsletter*, 1991, **4**(3), 4–5.
31 I. S. Whitaker, C. Twine, M. J. Whitaker, M. Welck, C. S. Brown and A. Shandall, *Postgrad. Med. J.*, 2007, **83**(980), 409–413.
32 C.-X. Zhang, X.-D. Tang and J.-A. Cheng, *Entomol. Res.*, 2008, **38**, S38–S47.
33 M. Berenbaum, *Bugs in the system: insects and their impact on human affairs*, Addison-Wesley: Reading, MA, 1995.
34 H. B. Weiss, *J. New York Entomol. Soc.*, 1946, **54**, 166.
35 D. Zimian, Z. Yonghua and G. Xiwu, *Ecol. Food Nutr.*, 1997, **36**(2–4), 209–220.
36 H. Balfour, *Man*, 1915, **15**, 17.
37 A. Bailey, C. Boden, J. Siberry and J. Summerhayes, 'Initiation rituals', in *Taboo* (Season 2, Episode 1), National Geographic Channel (USA), 2007.
38 V. Haddad Junior, J. L. Cardoso and R. H. Moraes, *Rev. Inst. Med. Trop. Sao Paulo*, 2005, **47**(4), 235–238.
39 J. Tremaine, 'Brazil', in *Wildboyz* (Season 2, Episode 2), MTV (Finland), 2004.
40 V. C. Clark, C. J. Raxworthy, V. Rakotomalala, P. Sierwald and B. L. Fisher, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**(33), 11617–11622.
41 J. W. Daly, T. F. Spande and H. M. Garraffo, *J. Nat. Prod.*, 2005, **68**(10), 1556–1575.
42 T. F. Spande, P. Jain, H. M. Garraffo, L. K. Pannell, H. J. C. Yeh, J. W. Daly, S. Fukumoto, K. Imamura, T. Tokuyama, J. A. Torres, R. R. Snelling and T. H. Jones, *J. Nat. Prod.*, 1999, **62**(1), 5–21.
43 A. Gurib-Fakim, *Mol. Aspects Med.*, 2006, **27**(1), 1–93.
44 P. Laurent, J. C. Braekman and S. Daloze, *Top. Curr. Chem.*, 2005, **240**, 167–229.
45 S. Trowell, *The Futurist*, 2003, **37**(1), 17–19.
46 J. D. Phillipson, *Phytochemistry*, 2007, **68**(22–24), 2960–2972.
47 E. Rodriguez and D. A. Levin, 'Biochemical parallelisms of repellents and attractants in higher plants and arthropods', in *Recent Advances in Phytochemistry*, ed. J. W. Wallace and R. L. Mansell, Plenum Press: New York, 1976, vol. 10, pp. 214–270.
48 E. M. Costa-Neto, *Sustain. Dev.*, 2004, **12**(3), 161–174.
49 Y. C. Yoo, B. H. Shin, J. H. Hong, J. Lee, H. Y. Chee, K. S. Song and K. B. Lee, *Arch. Pharm. Res.*, 2007, **30**(3), 361–365.
50 A. Gottlieb, *Sex, drugs and aphrodisiacs*, Ronin Publishing: Berkeley, California, 1993.
51 B. E. Read, *Peking Nat. Hist. Bull.*, 1935, **94**, 8–85.
52 R. A. Sherman, M. J. Hall and S. Thomas, *Annu. Rev. Entomol.*, 2000, **45**, 55–81.
53 D. J. Son, J. W. Lee, Y. H. Lee, H. S. Song, C. K. Lee and J. T. Hong, *Pharmacol. Ther.*, 2007, **115**(2), 246–270.
54 F. S. Ferreira, S. V. Brito, S. C. Ribeiro, A. A. Saraiva, W. O. Almeida and R. R. Alves, *BMC Complement. Altern. Med.*, 2009, **9**, 17.
55 M. Viuda-Martos, Y. Ruiz-Navajas, J. Fernandez-Lopez and J. A. Perez-Alvarez, *J. Food Sci.*, 2008, **73**(9), R117–R124.
56 S. E. Efem, K. T. Udoh and C. I. Iwara, *Infection*, 1992, **20**(4), 227–229.
57 O. Koru, F. Toksoy, C. H. Acikel, Y. M. Tunca, M. Baysallar, A. Uskudar Guclu, E. Akca, A. Ozkok Tuylu, K. Sorkun, M. Tanyuksel and B. Salih, *Anaerobe*, 2007, **13**(3–4), 140–145.
58 V. Bankova, R. Christov, A. Kujumgiev, M. C. Marcucci and S. Popov, *Z. Naturforsch. C*, 1995, **50**(3–4), 167–172.
59 M. C. B. Moraes, M. Pareja, R. A. Laumann and M. Borges, *Neotrop. Entomol.*, 2008, **37**(5), 489–505.
60 J. Ramos-Elorduy, E. Motte-Florae and J. M. Pino, 'Chinches medicinales de México (Insecta: Hemiptera)', in *Memorias del 15 Congreso Internacional de Medicina Tradicional y Alternativas Terapeuticaś*, Academia Mexicana de Medicina Tradicional: Ciudad de México, 2001, p. 31.
61 S. Haeder, R. Wirth, H. Herz and D. Spiteller, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**(12), 4742–4746.
62 T. M. F. Antonio, *The Food Insects Newsletter*, 1994, **7**(3), 3–4 (discussed in ref. 2).
63 J. O. Fasoranti, *The Food Insects Newsletter*, 1997, **10**(2), 1–5.
64 K. J. Mbata, *The Food Insects Newsletter*, 1991, **12**(2), 1–7.
65 S. K. Srivastava, N. Babu and H. Pandey, *Indian J. Tradit. Knowledge*, 2009, **8**(4), 485–494.

66 J. G. Kim, *Illustrated Drugs Encyclopedia* (in Korean), Nam San Dang: Seoul, South Korea, 1984.
67 Z. Huang. *Insects in Chinese culture*, http://www.cyberbee.net/~huang/pub/insect.html.
68 B. E. Read and A. Y. C.-M. Li, 'Insect drugs', in *Chinese Materia Medica* (translations, numbered in a single series, of ch. 39–52 of *Pen-ts'ao kang-mu*), ed. B. E. Read, Peking Natural History Bulletin: Peiping, China, 1941, vol. 1, pp. 1–101.
69 B. E. Read and S. Li, Chinese Materia Medica, Peking Natural History Bulletin: Peiping, China, 1931.
70 R. Rauh, S. Kahl, H. Boechzelt, R. Bauer, B. Kaina and T. Efferth, *Chin. Med.*, 2007, **2**, 8.
71 R. H. Boyle, The joy of cooking insects, Audubon, 1992, pp. 100–103.
72 R. Akre, *The Food Insects Newsletter*, 1993, **6**(1), 4.
73 Y. C. Chen and R. D. Alue, *The Food Insects Newsletter*, 1994, **7**(2), 1 and 8–10.
74 D. Zhang, X. Shen, Y. Wang, H. Wu and X. Mei, 'Studies on anti-inflammation and pain easing of the weaver ant', in *Ants and Rheumatoid Arthritis*, ed. Z. Wu, Jiangsu Scientific and Technological Publishing House, 1991, pp. 136–146.
75 Y. Zhao, J. Wang, P. Xie and S. Pan, *Guangxi Chin. Med.*, 1983, **6**(6), 39–43.
76 S. L. Tan, Y. He, Y. Huang and M. Gale Jr, *Curr. Opin. Pharmacol.*, 2004, **4**(5), 465–470.
77 Z. Wang, J. Nan, Z. Yang and Y. Shen, *J. Geratol.*, 1987, **7**(4), 41–44.
78 T. A. K. Prescott, J. Bramham, O. Zompro and S. K. Maciver, *Biochem. Syst. Ecol.*, 2009, **37**(6), 759–760.
79 R. N. Tharanathan and F. S. Kittur, *Crit. Rev. Food. Sci. Nutr.*, 2003, **43**(1), 61–87.
80 W. G. Goodman, *The Food Insects Newsletter*, 1989, **2, (3)**(1), 6.
81 V. P. Sharma, *Uttar Pradesh J. Zool.*, 1990, **10**(2), 133–136.
82 M. R. Berenbaum, 'The chemistry of defense: Theory and practice', in *Chemical Ecology: The Chemistry of Biotic Interaction*, ed. T. Eisner and J. Meinwald, National Academy Press: Washington, D.C., 1995, pp. 1–16.
83 V. Bankova, *Fitoterapia*, 1999, **70**(2), 190–193.
84 A. Kujumgiev, I. Tsvetkova, Y. Serkedjieva, V. Bankova, R. Christov and S. Popov, *J. Ethnopharmacol.*, 1999, **64**(3), 235–240.
85 K. Dettner, *Annu. Rev. Entomol.*, 1987, **32**, 17–48.
86 T. Eisner, *For love of insects*, Belknap Press of Harvard University Press: Cambridge, MA, 2003.
87 T. Eisner; M. Eisner and M. Siegler, *Secret weapons: defenses of insects, spiders, scorpions, and other many-legged creatures*. Belknap Press of Harvard University Press: Cambridge, MA, 2005.
88 J. Meinwald and T. Eisner, *Proc. Natl. Acad. Sci. U. S. A.*, 1995, **92**(1), 14–18.
89 M. S. Blum, *Chemical defenses of arthropods*, Academic Press: New York, 1981.
90 M. S. Blum, *Annu. Rev. Entomol.*, 1996, **41**, 353–374.
91 L. M. Roth and T. Eisner, *Annu. Rev. Entomol.*, 1962, **7**, 107.
92 J. M. Pasteels, J. C. Gregoire and M. Rowellrahier, *Annu. Rev. Entomol.*, 1983, **28**, 263–289.
93 C. Darwin, 'Life and letters', in *The Life and Letters of Charles Darwin* (1st edn), ed. F. Darwin, London, 1887, vol. 1, p. 50.
94 C. O. Schneider, *Rev. Chil. Hist. Nat.*, 1934, **38**, 44–46.
95 S. H. Scudder, *Psyche*, 1876, **1**(22), 137–140.
96 A. S. Packard, *J. New York Entomol. Soc.*, 1895, **3**(3), 110–127.
97 J. M. Pasteels, *Popul. Ecol.*, 2007, **49**(1), 5–14.
98 T. Piek, *Venoms of the Hymenoptera: Biochemical, pharmacological and behavioural aspects*, Academic: London, 1986.
99 T. Eisner; J. Meinwald, 'The chemistry of sexual selection', in *Chemical Ecology: The Chemistry of Biotic Interaction*, ed. T. Eisner and J. Meinwald, National Academy Press: Washington, D.C., 1995; pp. vii and 214.
100 S. Leclercq, J. C. Braekman, D. Daloze and J. M. Pasteels, *Fortschr. Chem. Org. Naturst.*, 2000, **79**, 115–229.
101 P. Laurent, J. C. Braekman, D. Daloze and J. Pasteels, *Eur. J. Org. Chem.*, 2003, **15**, 2733–2743.
102 J. Meinwald and T. Eisner, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**(12), 4539–4540.
103 O. Sticher, *Nat. Prod. Rep.*, 2008, **25**(3), 517–554.
104 A. Marston and K. Hostettmann, *Planta Med.*, 2009, **75**(7), 672–682.
105 J. L. Wolfender, *Planta Med.*, 2009, **75**(7), 719–734.
106 T. F. Molinski, *Curr. Opin. Drug Discovery*, 2009, **12**(2), 197–206.
107 W. W. Brey, A. S. Edison, R. E. Nast, J. R. Rocca, S. Saha and R. S. Withers, *J. Magn. Reson.*, 2006, **179**(2), 290–293.
108 T. F. Molinski, *Nat. Prod. Rep.*, 2010, **27**(3), 321–329.
109 F. E. Koehn, *Prog. Drug. Res.*, 2008, **65**(175), 177–210.
110 A. T. Dossey, S. S. Walse, J. R. Rocca and A. S. Edison, *ACS Chem. Biol.*, 2006, **1**(8), 511–514.
111 M. Gronquist, J. Meinwald, T. Eisner and F. C. Schroeder, *J. Am. Chem. Soc.*, 2005, **127**(31), 10810–10811.
112 A. T. Dossey, *Chemical biodiversity and signaling: Detailed analysis of FMRFamide-like neuropeptides and other natural products by NMR and bioinformatics* (dissertation), University of Florida: Gainesville, 2006.
113 Y. Tu, C. Jeffries, H. Ruan, C. Nelson, D. Smithson, A. A. Shelat, K. M. Brown, X. C. Li, J. P. Hester, T. Smillie, I. A. Khan, L. Walker, K. Guy and B. Yan, *J. Nat. Prod.*, 2010, **73**(4), 751–754.
114 T. Eisner and J. Meinwald, *Chemical Ecology: The Chemistry of Biotic Interaction*, National Academy Press: Washington, D.C., 1995.
115 P. D. Brock, *Phasmida* species file online, http://Phasmida.SpeciesFile.org (accessed 11th August 2010).
116 U. Carlberg, *Zool. Anzeiger*, 1986, **216**(1–2), 1–18.
117 D. Otte and P. Brock, *Phasmida* species file, Insect Diversity Association, Academy of Natural Sciences: Philadelphia, PA, 2005.
118 *Phasmid Study Group Web Page*, Phasmid Study Group (PSG): UK, 1979–present, http://phasmid-study-group.org.
119 E. H. Tilgner, *Systematics of Phasmida* (PhD thesis), University of Georgia: Athens, GA, 2002.
120 P. D. Brock, *The amazing world of stick and leaf-insects*, Amateur Entomologists Society, 1999.
121 I. Amato, *Chem. Eng. News*, 2006, **84**(39), 15.
122 G. O. Bedford, *Annu. Rev. Entomol.*, 1978, **23**, 125–149.
123 P. Bouchard, C. C. Hsiung and V. A. Yaylayan, *J. Chem. Ecol.*, 1997, **23**(8), 2049–2057.
124 U. Carlberg, *J. Chem. Ecol.*, 1981, **7**(5), 905–906.
125 U. Carlberg, *Zool. Anzeiger*, 1985, **215**(3–4), 177–188.
126 U. Carlberg, *Zool. Anzeiger*, 1985, **214**(3–4), 185–192.
127 U. Carlberg, *Zool. Anzeiger*, 1986, **217**(1–2), 31–38.
128 U. Carlberg, *Zool. Anzeiger*, 1987, **218**(5–6), 369–373.
129 Y. S. Chow and Y. M. Lin, *J. Entomol. Sci.*, 1986, **21**(2), 97–101.
130 A. T. Dossey, *ACS Chem. Biol.*, 2006, **1**(8), 473.
131 A. T. Dossey, M. Gottardo, J. M. Whitaker, W. R. Roush and A. S. Edison, *J. Chem. Ecol.*, 2009, **35**(8), 861–870.
132 A. T. Dossey, S. S. Walse, O. V. Conle and A. S. Edison, *J. Nat. Prod.*, 2007, **70**(8), 1335–1338.
133 A. T. Dossey, S. S. Walse and A. S. Edison, *J. Chem. Ecol.*, 2008, **34**(5), 584–590.
134 T. Eisner, *Science*, 1965, **148**(3672), 966.
135 T. Eisner, R. C. Morgan, A. B. Attygalle, S. R. Smedley, K. B. Herath and J. Meinwald, *J. Exp. Biol.*, 1997, **200**(19), 2493–2500.
136 G. M. Happ, J. D. Strandbe and C. M. Happ, *J. Morphol.*, 1966, **119**(2), 143.
137 H. Y. Ho and Y. S. Chow, *J. Chem. Ecol.*, 1993, **19**(1), 39–46.
138 A. D. Kinghorn, 2007 Arthur E. Schwarting and Jack L. Beal Award winners, *ASP Newsletter*, 2008.
139 J. Meinwald, M. S. Chadha, J. J. Hurst and T. Eisner, *Tetrahedron Lett.*, 1962, **1**, 29–33.
140 G. Schmeda-Hirschmann, *Z. Naturforsch. C*, 2006, **61**(7–8), 592–594.
141 R. M. Smith, J. J. Brophy, G. W. K. Cavill and N. W. Davies, *J. Chem. Ecol.*, 1979, **5**(5), 727–735.
142 M. C. Thomas, *Division of Plant Industry – Entomology Circular*, Florida Department of Agriculture & Consumer Services, 2001, p. 408.
143 R. Burks, *Chem. Eng. News*, 2007, **85**(33), 44.
144 G. A. Gangrade, *Curr. Sci. India*, 1964, **33**(23), 717–718.
145 J. Meinwald, G. M. Happ, J. Labows and T. Eisner, *Science*, 1966, **151**(3706), 79.
146 R. O. Albert, *Entomol. News*, 1947, **58**, 57–59.
147 J. Dziezyc, *J. Am. Vet. Med. Assoc.*, 1992, **200**(12), 1969.
148 E. A. Paysse, S. Holder and D. K. Coats, *Ophthalmology*, 2001, **108**(1), 190–191.
149 O. V. Conle, F. H. Hennemann and A. T. Dossey, *Ann. Entomol. Soc. Am.*, 2009, **102**(2), 210–232.

150 D. N Attenborough, 'Insects', in *Life*, BBC One (UK), 2009, 12th October–14th December 2009.
151 B. Wang, A. T. Dossey, S. S. Walse, A. S. Edison and K. M. Merz Jr, *J. Nat. Prod.*, 2009, **72**(4), 709–713.
152 F. Zhang, A. T. Dossey, C. Zachariah, A. S. Edison and R. Bruschweiler, *Anal. Chem.*, 2007, **79**(20), 7748–7752.
153 M. P. McLeod, A. T. Dossey and M. K. Ahmed, *Spectrosc. Int. J.*, 2007, **21**(3), 169–176.
154 C.-C. Hsiung, *J. Orthoptera Res.*, 2007, **16**(2), 207–221.
155 J. A. Elegbede, R. Flores and R. C. Wang, *Life Sci.*, 2003, **73**(22), 2831–2840.
156 J. Fernandes, C. O. da Fonseca, A. Teixeira and C. R. Gattass, *Oncol. Rep.*, 2005, **13**(5), 943–947.
157 L. Yeruva, C. Hall, J. A. Elegbede and S. W. Carper, *Anticancer Drugs*, 2009, **257**(2), 216–226.
158 L. Yeruva, K. J. Pierre, A. Elegbede, R. C. Wang and S. W. Carper, *Cancer Lett.*, 2007, **257**(2), 216–226.
159 N. Akiyama, M. Hijikata, A. Kobayashi, T. Yamori, T. Tsuruo and S. Natori, *Anticancer Res.*, 2000, **20**(1A), 357–362.
160 C. G. Azzoli, V. A. Miller, K. K. Ng, L. M. Krug, D. R. Spriggs, W. P. Tong, E. R. Riedel and M. G. Kris, *Cancer Chemother. Pharmacol.*, 2003, **51**(6), 493–498.
161 H. H. Bailey, D. Levy, L. S. Harris, J. C. Schink, F. Foss, P. Beatty and S. Wadler, *Gynecol. Oncol.*, 2002, **85**(3), 464–468.
162 G. R. Hudes, C. E. Szarka, A. Adams, S. Ranganathan, R. A. McCauley, L. M. Weiner, C. J. Langer, S. Litwin, G. Yeslow, T. Halberr, M. Qian and J. M. Gallo, *Clin. Cancer Res.*, 2000, **6**(8), 3071–3080.
163 G. Liu, K. Oettel, H. Bailey, L. V. Ummersen, K. Tutsch, M. J. Staab, D. Horvath, D. Alberti, R. Arzoomanian, H. Rezazadeh, J. McGovern, E. Robinson, D. DeMets and G. Wilding, *Invest. New Drugs*, 2003, **21**(3), 367–372.
164 S. M. Meadows, D. Mulkerin, J. Berlin, H. Bailey, J. Kolesar, D. Warren and J. P. Thomas, *Int. J. Gastrointest. Cancer*, 2002, **32**(2–3), 125–128.
165 S. P. Stratton, D. S. Alberts, J. G. Einspahr, P. M. Sagerman, J. A. Warneke, C. Curiel-Lewandrowski, P. B. Myrdal, K. L. Karlage, B. J. Nickoloff, C. Brooks, K. Saboda, M. L. Yozwiak, M. F. Krutzsch, C. Hu, M. Lluria-Prevatt, Z. Dong, G. T. Bowden and P. H. Bartels, *Cancer Prev. Res.*, 2010, **3**(2), 160–169.
166 C. O. da Fonseca, G. Schwartsmann, J. Fischer, J. Nagel, D. Futuro, T. Quirico-Santos and C. R. Gattass, *Surg. Neurol.*, 2008, **70**(3), 259–266; discussion, 266–267.
167 G. H. Ripple, M. N. Gould, J. A. Stewart, K. D. Tutsch, R. Z. Arzoomanian, D. Alberti, C. Feierabend, M. Pomplun, G. Wilding and H. H. Bailey, *Clin. Cancer Res.*, 1998, **4**(5), 1159–1164.
168 B. K. Feld, J. M. Pasteels and W. Boland, *Chemoecology*, 2001, **11**(4), 191–198.
169 J. Kuhn, E. M. Pettersson, B. K. Feld, L. H. Nie, K. Tolzin-Banasch, S. M. M'Rabet, J. Pasteels and W. Boland, *J. Chem. Ecol.*, 2007, **33**(1), 5–24.
170 M. Kunert, A. Soe, S. Bartram, S. Discher, K. Tolzin-Banasch, L. Nie, A. David, J. Pasteels and W. Boland, *Insect Biochem. Mol. Biol.*, 2008, **38**(10), 895–904.
171 N. J. Oldham, M. Veith, W. Boland and K. Dettner, *Naturwissenschaften*, 1996, **83**(10), 470–473.
172 R. J. Elzinga, *Fundamentals of entomology* (6th edn), Pearson/Prentice Hall: Upper Saddle River, NJ, 2004.
173 L. Collins and R. H. Scheffrahn, *Solenopsis invicta*, http://creatures.ifas.ufl.edu/urban/ants/red_imported_fire_ant.htm.
174 L. Chen and H. Y. Fadamiro, *Toxicon*, 2009, **53**(5), 479–86.
175 C. S. Lofgren, W. A. Banks and B. M. Glancey, *Annu. Rev. Entomol.*, 1975, **20**, 1–30.
176 J. G. MacConnell, M. S. Baum and H. M. Fales, *Science*, 1970, **168**(3933), 840.
177 J. G. MacConnell, M. S. Blum and H. M. Fales, *Tetrahedron*, 1971, **27**(6), 1129.
178 T. H. Jones, H. L. Voegtle, H. M. Miras, R. G. Weatherford, T. F. Spande, H. M. Garraffo, J. W. Daly, D. W. Davidson and R. R. Snelling, *J. Nat. Prod.*, 2007, **70**(2), 160–168.
179 D. J. Newman and G. M. Cragg, *J. Nat. Prod.*, 2007, **70**(3), 461–477.
180 J. Y. Ortholand and A. Ganesan, *Curr. Opin. Chem. Biol.*, 2004, **8**(3), 271–280.
181 H. E. Evans, *Life on a little-known planet: A biologist's view of insects and their world*, The Lyons Press: New York, 1993.
182 C. Ramos-Elorduyd, J. Moreno and J. M. Pino, *J. Ethnobiol.*, 1988, **8**(2), 195–202.
183 G. R. Pettit, *J. Nat. Prod.*, 1996, **59**(8), 812–821.
184 G. R. Pettit, J. L. Hartwell and H. B. Wood, *Cancer Res.*, 1968, **28**(10), 2168–2169.
185 G. R. Pettit, Q. Ye, D. L. Herald, F. Hogan and R. K. Pettit, *J. Nat. Prod.*, 2010, **73**(2), 164–166.
186 J.-L. Dimarcq and a. Hunneyball, *Drug Discovery Today*, 2003, **8**(3), 107–110.
187 *Non-Wood News*, Forestry Department of the Food and Agriculture Organization of the United Nations, 1995, vol. 2.
188 *Non-Wood News*, Forestry Department of the Food and Agriculture Organization of the United Nations, 1996, vol. 3.
189 M. Slocinska, P. Marciniak and G. Rosinski, *Protein Pept. Lett.*, 2008, **15**(6), 578–585.
190 P. Bulet and R. Stocklin, *Protein Pept. Lett.*, 2005, **12**(1), 3–11.
191 V. C. A. Matanic and V. Castilla, *Int. J. Antimicrob. Agents*, 2004, **23**(4), 382–389.
192 D. C. de Graaf, M. Aerts, M. Brunain, C. A. Desjardins, F. J. Jacobs, J. H. Werren and B. Devreese, *Insect Mol. Biol.*, 2010, **19**(Suppl. 1), 11–26.
193 R. Ylitalo, S. Lehtinen, E. Wuolijoki, P. Ylitalo and T. Lehtimaki, *Arzneimittelforschung*, 2002, **52**(1), 1–7.
194 J. Ramos-Elorduy, E. Motte-Florae, J. M. Pino and C. Andary, 'Les insectes utilises en medicine traditionnelle au Mexique: Perspectives', in *Healing: Yesterday, Today, and Tomorrow* (CD ROM), Erga Edizioney: Geneva, 1999, pp. 271–290.
195 C. Andary, E. Motte-Florae, J. Ramos-Elorduy and A. Privat, 'Chemical screening: Updated methodology applied to medicinal insects', *The 3rd European Colloquium on Ethnopharmacology and 1st International Conference on Anthropology and History of Health and Disease*, Erga Edizioness, 1996.
196 K. Konno, M. Hisada, Y. Itagaki, H. Naoki, N. Kawai, A. Miwa, T. Yasuhara and H. Takayama, *Biochem. Biophys. Res. Commun.*, 1998, **250**(3), 612–616.
197 J. W. Fox and S. M. T. Serrano, *Curr. Pharm. Des.*, 2007, **13**(28), 2927–2934.
198 H. F. Lodish, *Molecular cell biology* (4th edn), W. H. Freeman: New York, 2000.
199 T. H. Jones, D. A. Clark, B. E. Heterick and R. R. Snelling, *J. Nat. Prod.*, 2003, **66**(3), 325–326.
200 M. L. Oldfield, The value of conserving genetic resources, *U.S. Department of the Interior*, National Park Service: Washington D.C., 1984.
201 M. L. Oldfield, *The value of conserving genetic resources*, Sinauer Associates, Inc.: Sunderland, Massachusetts, 1989.
202 M. Sagawa, T. Nakazato, H. Uchida, Y. Ikeda and M. Kizaki, *Cancer Sci.*, 2008, **99**(9), 1820–1826.
203 J. A. Sakoff, S. P. Ackland, M. L. Baldwin, M. A. Keane and A. McCluskey, *Invest. New Drugs*, 2002, **20**(1), 1–11.
204 L. N. Andrade, T. M. de Lima, R. Curi and A. M. Castrucci, *Toxicol. In Vitro*, 2005, **19**(4), 553–560.
205 J. Y. Kong and S. W. Rabkin, *Prostaglandins, Leukotrienes, Essential Fatty Acids*, 2002, **67**(5), 293–302.
206 J. M. Brand, M. S. Blum and H. H. Ross, *Insect Biochem.*, 1973, **3**(9), 45–51.
207 J. L. Arbiser, T. Kau, M. Konar, K. Narra, R. Ramchandran, S. A. Summers, C. J. Vlahos, K. Ye, B. N. Perry, W. Matter, A. Fischl, J. Cook, P. A. Silver, J. Bain, P. Cohen, D. Whitmire, S. Furness, B. Govindarajan and J. P. Bowen, *Blood*, 2007, **109**(2), 560–565.
208 G. B. Yi, D. McClendon, D. Desaiah, J. Goddard, A. Lister, J. Moffitt, R. K. Meer, R. deShazo, K. S. Lee and R. W. Rockhold, *Int. J. Toxicol.*, 2003, **22**(2), 81–86.
209 G. Howell, J. Butler, R. D. deShazo, J. M. Farley, H. L. Liu, N. P. D. Nanayakkara, A. Yates, G. B. Yi and R. W. Rockhold, *Ann. Allerg. Asthma Immunol.*, 2005, **94**(3), 380–386.
210 G. R. Pettit, Y. H. Meng, D. L. Herald, J. C. Knight and J. F. Day, *J. Nat. Prod.*, 2005, **68**(8), 1256–1258.
211 J. Polonsky, Z. Varon, H. Jacquemin and G. R. Pettit, *Experientia*, 1978, **34**(9), 1122–1123.
212 G. R. Pettit and R. H. Ode, *Lloydia*, 1976, **39**(2–3), 129–133.
213 G. R. Pettit, R. M. Blazer and D. A. Reierson, *Lloydia*, 1977, **40**(3), 247–252.

214 W. E. Kunin and J. H. Lawton, 'Does biodiversity matter? Evaluating the case for conserving species', in *Biodiversity: A Biology of Numbers and Differences*, ed. K. J. Gaston, Blackwell Science: Oxford, 1996, pp. 283–308.
215 G. R. Pettit, L. E. Houghton, N. H. Rogers, R. M. Coomes, D. F. Berger, P. R. Reucroft, J. F. Day, J. L. Hartwell and H. B. Wood Jr, *Experientia*, 1972, **28**(4), 381–382.
216 J. Y. Leem, C. Nishimura, S. Kurata, I. Shimada, A. Kobayashi and S. Natori, *J. Biol. Chem.*, 1996, **271**(23), 13573–13577.
217 H. Kawada, M. Kojima, T. Kimura, S. Natori, K. Sasaki and H. Sasaki, *Jpn. J. Ophthalmol.*, 2009, **53**(5), 531–535.
218 N. Akiyama and S. Natori, *Cancer Sci.*, 2003, **94**(4), 400–404.
219 Y. Fujiwara, M. Mangetsu, P. Yang, H. Kofujita, K. Suzuki, Y. Ohfune and T. Shinada, *Biol. Pharm. Bull.*, 2008, **31**(4), 722–725.
220 M. Pavan and G. Bo, *Physiol. Comput. Oecol.*, 1953, **3**, 307–312.
221 J. Piel, D. Butzke, N. Fusetani, D. Hui, M. Platzer, G. Wen and S. Matsunaga, *J. Nat. Prod.*, 2005, **68**(3), 472–479.
222 G. Estrada, E. Villegas and G. Corzo, *Nat. Prod. Rep.*, 2007, **24**(1), 145–161.
223 F. C. Schroeder, A. E. Taggi, M. Gronquist, R. U. Malik, J. B. Grant, T. Eisner and J. Meinwald, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**(38), 14283–14287.
224 C. A. Olsen, I. R. Mellor, P. Wellendorph, P. N. Usherwood, M. Witt, H. Franzyk and J. W. Jaroszewski, *ChemMedChem*, 2006, **1**(3), 303–305.
225 K. Nakanishi, C. Seok-Ki, D. Hwang, K. Lerro, M. Orlando, A. G. Kalivrentenos, A. Eldefrawi, M. Eldefrawi and P. N. R. Usherwood, *Pure Appl. Chem.*, 1994, **66**(4), 671–678.
226 A. T. Eldefrawi, M. E. Eldefrawi, K. Konno, N. A. Mansour, K. Nakanishi, E. Oltz and P. N. Usherwood, *Proc. Natl. Acad. Sci. U. S. A.*, 1988, **85**(13), 4910–4913.
227 L. S. Jensen, U. Bolcho, J. Egebjerg and K. Stromgaard, *ChemMedChem*, 2006, **1**(4), 419–428.
228 E. Szolajska, J. Poznanski, M. L. Ferber, J. Michalik, E. Gout, P. Fender, I. Bailly, B. Dublet and J. Chroboczek, *Eur. J. Biochem.*, 2004, **271**(11), 2127–2136.
229 S. R. Johnson, J. A. Copello, M. S. Evans and A. V. Suarez, *Toxicon*, 2010, **55**(4), 702–710.
230 K. Meylaers, A. Cerstiaens, E. Vierstraete, G. Baggerman, C. W. Michiels, A. De Loof and L. Schoofs, *Curr. Pharm. Des.*, 2003, **9**(2), 159–174.
231 A. J. Beattie, C. L. Turnbull, T. Hough and R. B. Knox, *Ann. Entomol. Soc. Am.*, 1986, **79**(3), 448–450.
232 A. Bexfield, Y. Nigam, S. Thomas and N. A. Ratcliffe, *Microbes Infect.*, 2004, **6**(14), 1297–1304.
233 H. G. Boman, *Immunol. Rev.*, 2000, **173**, 5–16.
234 H. G. Boman, I. Faye, G. H. Gudmundsson, J. Y. Lee and D. A. Lidholm, *Eur. J. Biochem.*, 1991, **201**(1), 23–31.
235 H. G. Boman and D. Hultmark, *Annu. Rev. Microbiol.*, 1987, **41**, 103–126.
236 V. Cerovsky, J. Zdarek, V. Fucik, L. Monincova, Z. Voburka and R. Bem, *Cell. Mol. Life Sci.*, 2009, **67**(3), 455–466.
237 S. Chernysh, S. I. Kim, G. Bekker, V. A. Pleskach, N. A. Filatova, V. B. Anikin, V. G. Platonov and P. Bulet, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**(20), 12628–12632.
238 S. Cociancich, P. Bulet, C. Hetru and J. A. Hoffmann, *Parasitol. Today*, 1994, **10**(4), 132–139.
239 J. Hoffmann, *Nat. Immunol.*, 2007, **8**(6), 543–545.
240 L. Huberman, N. Gollop, K. Y. Mumcuoglu, C. Block and R. Galun, *J. Wound Care*, 2007, **16**(3), 123–127.
241 L. Huberman, N. Gollop, K. Y. Mumcuoglu, E. Breuer, S. R. Bhusare and Y. Shai, *Med. Vet. Entomol.*, 2007, **21**(2), 127–131.
242 D. Hultmark, A. Engstrom, K. Andersson, H. Steiner, H. Bennich and H. G. Boman, *EMBO J.*, 1983, **2**(4), 571–576.
243 D. Hultmark, H. Steiner, T. Rasmuson and H. G. Boman, *Eur. J. Biochem.*, 1980, **106**(1), 7–16.
244 A. Kerridge, H. Lappin-Scott and J. R. Stevens, *Med. Vet. Entomol.*, 2005, **19**(3), 333–337.
245 J. Y. Leem, I. J. Jeong, K. T. Park and H. Y. Park, *FEBS Lett.*, 1999, **442**(1), 53–56.
246 K. Meylaers, E. Clynen, D. Daloze, A. DeLoof and L. Schoofs, *Insect Biochem. Mol. Biol.*, 2004, **34**(1), 43–49.
247 S. Natori, *Ciba Foundation Symp.*, 1994, **186**, 123–132.
248 A. Pillai, S. Ueno, H. Zhang, J. M. Lee and Y. Kato, *Biochem. J.*, 2005, **390**, 207–214.
249 O. Toke, *Biopolymers*, 2005, **80**(6), 717–735.
250 M. Wachinger, A. Kleinschmidt, D. Winder, N. von Pechmann, A. Ludvigsen, M. Neumann, R. Holle, B. Salmons, V. Erfle and R. Brack-Werner, *J. Gen. Virol.*, 1998, **79**(4), 731–740.
251 D. Wade, D. Andreu, S. A. Mitchell, A. M. V. Silveira, A. Boman, H. G. Boman and R. B. Merrifield, *Int. J. Pept. Prot. Res.*, 1992, **40**(5), 429–436.
252 H. Steiner, D. Hultmark, A. Engstrom, H. Bennich and H. G. Boman, *Nature*, 1981, **292**(5820), 246–248.
253 A. M. Aerts, I. E. Francois, B. P. Cammue and K. Thevissen, *Cell. Mol. Life Sci.*, 2008, **65**(13), 2069–2079.
254 A. Vilcinskas and J. Gross, *J. Pest Sci.*, 2005, **78**(4), 187–191.
255 D. Ricci, D. Fraternale, L. Giamperi, A. Bucchini, F. Epifano, G. Burini and M. Curini, *J. Ethnopharmacol.*, 2005, **98**(1–2), 195–200.
256 B. Altincicek and A. Vilcinskas, *Insect Mol. Biol.*, 2009, **18**(1), 119–125.
257 M. J. Ryu, V. Anikin, S. H. Hong, H. Jeon, Y. G. Yu, M. H. Yu, S. Chernysh and C. Lee, *Mol. Cell. Biochem.*, 2008, **313**(1–2), 91–102.
258 M. Friedman, P. R. Henika and R. E. Mandrell, *J. Food Prot.*, 2003, **66**(10), 1811–1821.
259 H. Schildknecht, *Endeavour*, 1971, **30**(111), 136.
260 J. Yang, M. Yamamoto, J. Ishibashi, K. Taniai and M. Yamakawa, *Eur. J. Biochem.*, 1998, **255**(3), 734–738.
261 N. Wilson-Rich, M. Spivak, N. H. Fefferman and P. T. Starks, *Annu. Rev. Entomol.*, 2009, **54**, 405–423.
262 E. De Clercq, *Med. Res. Rev.*, 2000, **20**(5), 323–349.
263 M. Wachinger, T. Saermark and V. Erfle, *FEBS Lett.*, 1992, **309**(3), 235–241.
264 R. Meenakshisundaram, S. Sweni and P. Thirumalaikolundusubramanian, *AIDS Res. Ther.*, 2009, **6**, 25.
265 A. Baghian, J. Jaynes, F. Enright and K. G. Kousoulas, *Peptides*, 1997, **18**(2), 177–183.
266 A. F. Esser, R. M. Bartholomew, F. C. Jensen and H. J. Mullereberhard, *Proc. Natl. Acad. Sci. U. S. A.*, 1979, **76**(11), 5843–5847.
267 H. M. Shen and V. Tergaonkar, *Apoptosis*, 2009, **14**(4), 348–363.
268 E. T. Wong and V. Tergaonkar, *Clin. Sci.*, 2009, **116**(6), 451–465.
269 C. Q. Ling, B. Li, C. Zhang, D. Z. Zhu, X. Q. Huang, W. Gu and S. X. Li, *Ann. Oncol.*, 2005, **16**(1), 109–115.
270 D. Winder, W. H. Gunzburg, V. Erfle and B. Salmons, *Biochem. Biophys. Res. Commun.*, 1998, **242**(3), 608–612.
271 M. J. Kim, S. J. Hong, J. Yang and H. K. Kim, *Neurol. Res.*, 2007, **29**(Suppl. 1), S72–S77.
272 K. Konno, H. Ono, M. Nakamura, K. Tateishi, C. Hirayama, Y. Tamura, M. Hattori, A. Koyama and K. Kohno, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**(5), 1337–1341.
273 J. J. Scott, D. C. Oh, M. C. Yuceer, K. D. Klepzig, J. Clardy and C. R. Currie, *Science*, 2008, **322**(5898), 63.
274 D. C. Oh, J. J. Scott, C. R. Currie and J. Clardy, *Org. Lett.*, 2009, **11**(3), 633–636.
275 M. Isaka, S. Palasarn, K. Sriklung and K. Kocharin, *J. Nat. Prod.*, 2005, **68**(11), 1680–1682.
276 T. Bunyapaiboonsri, S. Yoiprommarat, K. Intereya, P. Rachtawee, N. L. Hywel-Jones and M. Isaka, *J. Nat. Prod.*, 2009, **72**(4), 756–759.
277 A. Engstrom, P. Engstrom, Z. J. Tao, A. Carlsson and H. Bennich, *EMBO J.*, 1984, **3**(9), 2065–2070.
278 J. Lambert *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 1989, **86**(1), 262–266.
279 E. Haberman, *Science*, 1972, **177**(4046), 314–322.
280 L. Chen and H. Y. Fadamiro, *Toxicon*, 2009, **53**(5), 469–478.