Drug Discovery in Insects

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Insects: True Pioneers in Anti-Infective Therapy and What We Can Learn from Them**

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Since the introduction of anti-infectives more than 100 years ago[1] our world has changed: For the first time in human history we were able to combat life-threatening diseases caused by bacteria, fungi, viruses, and protozoa. At least in industrialized countries we are now so familiar with anti-infectives, especially antibiotics, that almost everybody has used them at least once to treat various bacterial infections, which otherwise would have at least taken longer to cure and would have been more painful and severe.

However, the golden age of anti-infectives that started in 1950 is coming to an end: Resistance against new anti-infectives, including those representing our last line of defense (e.g. for antibiotics), is emerging dramatically, leading to a return of almost eradicated diseases such as tuberculosis even in industrialized countries. New diseases (e.g. the recent outbreak of swine flu in Mexico or virus-induced severe acute respiratory syndrome, SARS) can spread as rapidly as we can travel, and global warming will allow diseases still regarded as tropical diseases to become endemic in temperate zones. These are only a few—always global—problems of anti-infective therapy. Additionally, as anti-infective research is very expensive and time consuming,[2] several pharmaceutical companies have reduced activity in this important area. These combined factors may lead to a dramatic situation in the very near future similar to that 100 years ago. Our only chance is to continue to fight the microorganisms in order to at least maintain the current status quo. We desperately need new anti-infectives!

As natural products, their derivatives, and compounds inspired by natural products have been by far the major source for clinically used anti-infectives,[3] one idea might be to revitalize natural products research as described previously.[4] Here, it might be especially fruitful to consider not only single, free-living organisms as was mainly done in the past (e.g. soil bacteria) but at complex biological systems that consist of (in some cases several) different organisms. With over a million estimated species, insects are the most diverse group of animals on earth and might actually represent 90% of all animal life forms on our planet.[19] Moreover, insects are an ancient class of arthropods; the first primitive members appeared almost 400 million years ago and spread to nearly all environments on our planet. Owing to this high diversity and because of the long time since the first appearance of insects, numerous microorganisms have adapted specifically to insects as hosts and/or food sources. Insects can thus be regarded as a huge reservoir for unusual microorganisms with potential biotechnological and/or pharmaceutical applications.[5]

Insects are known as a rich source of bacteria that produce interesting natural products,[6,7] as highlighted by *Paederus* bettle symbionts that produce pederin.[8] Moreover, recent reseach indicates that the concept of anti-infective therapy was established in insects millions of years ago: The first example of such a strategy is found in the European beewolf (*Philanthus triangulum*, Hymenoptera, Crabronidae), a solitary digger wasp that constructs nest burrows in sandy soil. Beewolf females catch and paralyze honeybees and use them as a food source for their larvae in these soil nests. The larvae feed on the bees and spin a cocoon in which they hibernate, until they metamorphose into a new generation of beewolfs the subsequent summer. The brood cell is humid and warm—ideal conditions not only for the larvae but also for the bacteria and fungi that live in the soil and thus could infect and kill the larvae. How are the larvae protected from infection during this long period of time? The answer to this question was presented by Kaltenpoth et al. in 2005:[9] After constructing the brood cell, beewolf females smear a white substance from their antennae onto the ceiling of the cell. This white substance is not less than antibiotic-producing *Streptomyces* bacteria,[10] which are "cultivated" for this purpose in specialized antennal glands. The deposition of the bacteria dramatically enhances the survival rate of the larvae and also protects the cocoon as the bacteria were also found in the silk. Unfortunately, the nature of the protective compound is yet unknown as the symbiont could not yet be cultivated.

The second example was reported by the groups led by Currie and Clardy, who investigated the southern pine beetle

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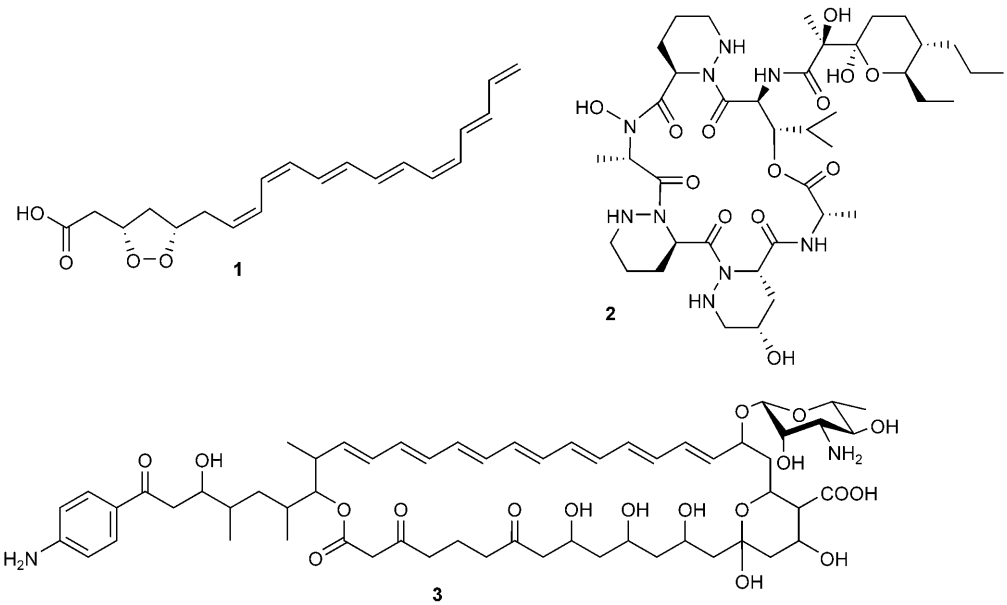
(*Dendroctonus frontalis*). Southern pine beetles can kill healthy pine trees by pheromone-guided mass attacks and therefore have considerable economic impact.[11] The beetles live in symbiosis with the fungus *Entomocorticium* sp. A, which serves as a food source for the beetle larvae. Adult beetles carry the fungus in a specialized storage compartment called the mycangium, and during excavation of ovipositional galleries within the inner bark of the tree they inoculate these galleries with the fungus. However, the antagonistic fungus *Ophiostoma minus*, which lives on *Tarsonemus* mites that themselves live on the beetle, can outcompete *Entomocorticium* sp. A and thereby disrupt larval development. To maintain the symbiosis between *D. frontalis* and *Entomocorticium* sp. A, the beetles carry a *Streptomyces* bacterium in the mycangium with high similarity to *S. thermosacchari*. Thus, the galleries are inoculated with the bacteria as well as by the fungal food source. The groups of Currie and Clardy could isolate the light-sensitive polyene peroxide mycangimycin (**1**) from the bacterium, which they could grow in standard *Streptomyces* medium.[12] Mycangimycin (**1**) was shown to efficiently inhibit *O. minus* while only slightly affecting *Entomocorticium* sp. A. It also inhibits human pathogenic *Candida albicans*, including amphotericin-resistant mutants.[13] The 1,2-dioxolane functionality of **1** is similar to pharmacophores with documented antimalarial activity such as the 1,2,4-trioxane unit in artemisinin, and therefore activity against *Plasmodium falciparum* was also tested. Here the activity of **1** was in the nanomole range, similar to clinically used antimalarial drugs.[13] Clearly the next steps could be the identification of the mode of action in fungi and protozoa as well as the generation of simplified and more stable analogues.

The third example is the highly evolved and very old symbiosis between fungus-growing ants and their fungi.[14] The probably best studied system here is that between leaf-cutter ants and their fungal cultivars. The ants carefully tend the fungus, which serves as their major food source; they feed it with fresh leaves and protect the fungal garden from microbial pathogens, including the specialized, highly virulent, and devastating fungi of the genus *Escovopsis*. So the question arises: How is the cultivar monoculture maintained and protected? Again, actinobacteria as well as *Burkholderia* sp.[15] have been identified. Already in 1999 Currie et al. identified a *Pseudonocardia* strain that could efficiently inhibit the growth of *Escovopsis*,[16] and very recently they, together with the Clardy group, could identify the responsible compound together. Only a single compound was produced by the *Pseudonocardia* strain, which proved to be the structurally and biochemically interesting new cyclic depsipeptide dentigerumycin (**2**).[17] It consists of the unusual amino acids piperazic acid, γ-hydroxypiperazic acid, β-hydroxyleucine, and *N*-hydroxyalanine; it also has a complex polyketide-derived side chain and a total of 12 stereogenic centers, all of which could be determined using a combination of different methods. Bioassays revealed that **2** clearly inhibits the pathogenic *Escovopsis* while the cultivar was resistant. Similar to the action of **1**, *C. albicans* and its amphotericin-resistant mutants were also inhibited by **2**.[17]

Recently the group of Spiteller could isolate *Streptomyces* sp. that was similar to *S. albidoflavus* or *S. griseus* from several different leaf-cutting ants and could show that they produce the macrolide candicidin D (**3**) as well as other candicidin derivatives.[18] At least one associated microorganism in all three leaf-cutting ant species analyzed was shown to produce these known antifungal compounds. Candicidin also showed very good activity against *Escovopsis* but was either not or only weakly active against other pathogenic fungi. As expected, the authors could also identify candicidin biosynthesis genes in the candicidin-producing *Streptomyces* strains. Candicidins are clinically used and highly active antifungals; they interact with sterols in the fungal cell membrane leading to K^+ leakage and subsequent cell death. As resistance against polyene macrolides has been rarely observed, the authors suggest that the leaf-cutting ants use a strategy similar



to that used by humans: *Escovopsis* simply cannot adapt quickly enough to these type of compounds.

Are these compounds and/or symbiotic systems merely interesting examples of chemical ecology and of the beauty of nature in general? Or do they also have implications in human anti-infective research or natural product research in general? I think the latter is the case, for three main reasons:

1. These examples are only the tip of the iceberg as many similar cases have not yet been investigated in detail. In examining closely related systems (other bark beetles, other fungus-growing ants) one can expect to find further compounds (some similar, some completely different), as the identification of candicidins and dentigerumycin has already shown.
2. One of the big problems of natural product research and drug discovery is that the quality of new sources of natural products as well as the quality of the natural products themselves cannot be predicted. A range of compounds are isolated from different sources and tested against different targets in order to find a useful activity. In the examples presented, the symbiotic and/or antagonistic relationship already suggests a biological activity, leading to a much more focused approach. In the future micro-organisms living in the soil might also be useful sources, but so far the biological and chemical complexity of soil and our lack of understanding of such a complex system prevent this. Although the described symbiotic systems are complex multipartite systems, they can be reconstituted and analyzed in detail in the lab.
3. These and related systems can be used to understand the formation and avoidance of resistance—probably the most important task in the immediate future. How can a symbiosis last for probably millions of years with the same or similar partners/compounds, and yet resistance against clinically used antibiotics develops within several years?

In summary, I am confident that several new natural products will be identified in the future if we are willing to continue this kind of research. Times have never been better as we now have excellent analytical (e.g. sensitive and affordable mass spectrometers) and molecular tools (e.g. affordable and fast whole-genome sequencing). Natural product research and drug discovery in general will clearly benefit from this work and from the analysis of "easy-to-study" and "simple" insect–bacteria symbioses.

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