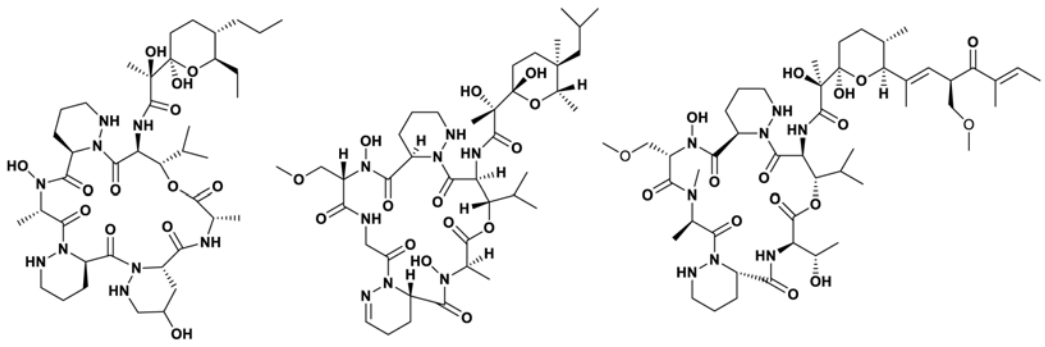
Hot Topics in Pharmacognosy: Where Does One Find Novel Antimicrobials Today?

By Dr. David Newman

As we all realize, the search for novel antimicrobials (antibacterial and antifungal agents in my lexicon) has now largely moved from "big pharma" into the realm of small pharma, non-profits and academia, exemplified by Cubist Pharmaceuticals and other companies of similar or smaller size, plus very interesting input from a number of academic consortia. In addition, the large predominately terrestrial microbial collections of yesteryears have been distributed to a number of smaller groups. For example, the Merck collection is now with a non-profit and the Lilly collection has been dispersed to a number of smaller companies. Two of the other main collections in the United States, those of Bristol Myers Squibb and Pfizer (first Lederle, then Wyeth), appear to be just stored and not utilized.

However, in the last 10 to 15 or so years, there have been some extremely interesting reports of studies related to antibacterial and antifungal agents that have been identified as a result of studying the interactions between insects, bacteria, and fungi. In addition, of course, there are increasing number of reports of antimicrobial agents coming from studies of shallow and deep sea microbes. These organisms are frequently isolated from "muds" and not necessarily from studies of commensals of invertebrates.

In the late 1990s, a series of papers from Dr. Cameron Currie's group[1, 2] identified the "apparently symbiotic relationship" between the leaf-cutter Attine ants in South America, their fungal gardens that they cultivated for food, and the production of a bacterial antifungal agent effective against a rapidly growing fungus identified as an *Escovopsis* species. This was originally thought to be a generalized example of tripartite symbiosis, and in the example that I will discuss below, it almost certainly is. However, the generalized example does not hold when extended to other published examples in this geographic area, as demonstrated by the excellent recent review by Dr. Ulrich Mueller,[3] a collaborator of Dr. Currie in some of the earliest studies. In the wider examples given by Dr. Mueller, a plethora of bacteria associated with the ant produce a number of both known and previously undescribed antifungal agents in response to the challenge by *Escovopsis* species in attacking the ants' fungal farms in other parts of the New World.



1. Dentigerumycin

2. Aurantimycin B

3. Azinothricin

However, in the following case, there is no doubt about the symbiosis. In 2009, ASP Fellow Dr. Jon Clardy working with Dr. Currie, published an extremely interesting paper[4] showing that the *Pseudonocardia* strain covering the ant body in this particular area in Panama, produced an antifungal agent directed against the attacker (*Escovopsis* sp.); however, this had little to no effect upon the basidiomycota that were the ant's food source. The compound, named as dentigerumycin (**1**) contained the unusual pyrazine amino acids that had been reported earlier in the anti-

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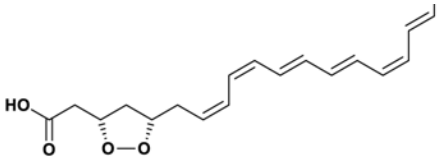
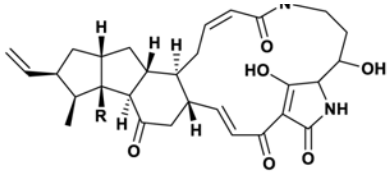
tumor aurantimycin and azinothricin molecules, but the arrangement of substituents within the depsipeptide rings differed (see structures **2, 3**).

Continuing the studies with the Currie and other related laboratories, the Clardy laboratory then turned their attention to the interplay between the southern pine beetle (SPB) and its microbes. The SPB has a mutualistic relationship with a species of the fungus *Entomocorticium*, which acts as a food source for the beetle larvae. However, there is a "hitch-hiker" in this process; a mite attaches itself to the chitin exoskeleton of the SPB and brings along another faster-growing fungus, *Ophiostoma minus*. Thus if this latter fungus is allowed to grow, the larvae lose their food source. In this case, from the rampant growth of actinomycetes observed in the system, two morphotypes were isolated and cultivated. One produced a novel antifungal agent named mycangimycin (**4**) which had the unusual peroxo substitution reminiscent of artemisinin, and it exhibited antiplasmodial activity comparable to this agent.[5] The other morphotype did not reveal any antifungal agent until a different metabolomics strategy was used. Then, a series of novel antifungal agents, the frontalamides (**5,6**), were isolated from this morphotype.[6]

These are just a few of the examples that have been published in the last few years, and for further information, the excellent reviews from the Clardy[7] and Hertweck[8] groups should be read. They demonstrate that we have not even brushed the surface of the possibilities for sourcing novel agents, particularly naturally occurring structures that demonstrate antifungal activity. Even today, physicians are still using amphotericin (aka "ampho-terrible") as a drug frequently of last resort. ■



4. Mycangimycin



5. Frontalamide A, R = OH
6. Frontalamide B, R = H

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REFERENCES

1. Currie, C. R. and Stuart, A. E. Weeding and grooming of pathogens in agriculture by ants. *Proc R Soc Lond B*, **2001**, 298, 1033-1039

2. Currie, C. R. A community of ants, fungi and bacteria: a multilateral approach to studying symbiosis. *Annu Rev Microbiol*, **2001**, 55, 357-380

3. Mueller, U. Symbiont recruitment versus ant-symbiont co-evolution in the attine ant-microbe symbiosis. *Curr Opin Microbiol*, **2012**, 15, 269-277

4. Oh, D.-C., Poulsen, M., Currie, C.. R. and Clardy, J. Dentigerumycin: a bacterial mediator of an ant-fungus symbiosis. *Nat Chem Biol,* **2009**, 5, 391-393

5. Oh, D.-C., Scott, J. J., Currie, C. R. and Clardy, J. Mycangimycin, a polyene peroxide from a mutualist *Streptomyces* sp. *Org Lett,* **2009**, 11, 633-636

6. Blodgett, J. A. V., Oh, D.-C., Cao, S., Currie, C. R. and Clardy, J. Common biosynthetic origins for polycyclic tetramate macrolactams from phylogenetically diverse bacteria. *Proc Natl Acad Sci USA*, **2010**, 107, 11692-11697

7. Scherlach, K., Graupner, K. and Hertweck, C. Molecular bacteria-fungi interactions: Effects on environment, food and medicine. *Annu Rev Microbiol,* **2013**, 67, 375-397

8. Amadhar, T. R., Beemelmanns, C., Currie, C. R. and Clardy, J. Bacterial symbionts in agricultural systems provide a strategic resource for antibiotic discovery. *J Antibiot*, **2014**, 67, 53-58