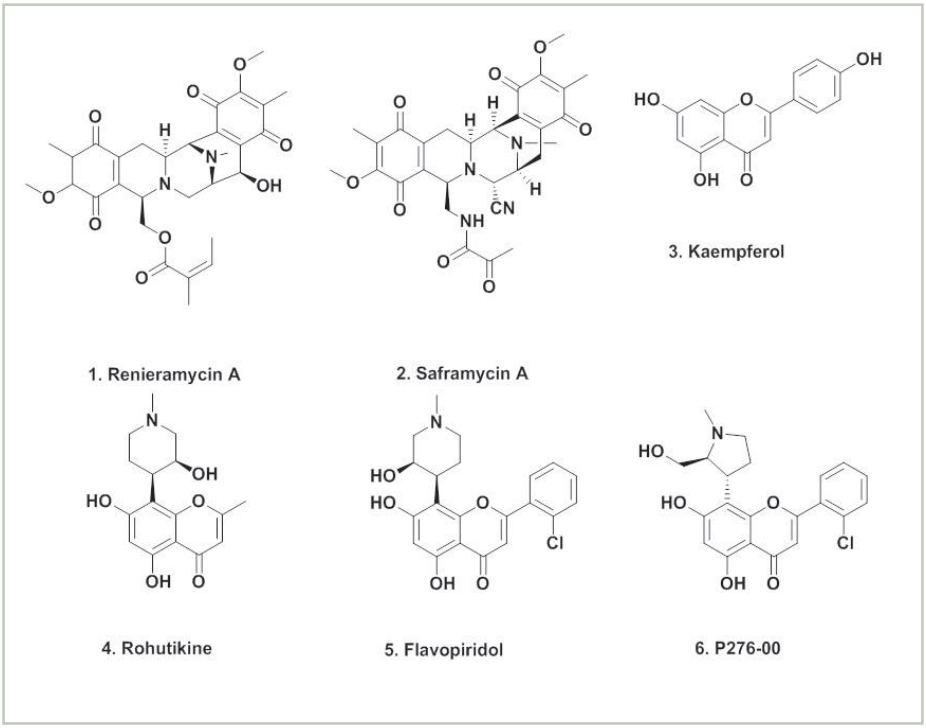
Hot Topics in Pharmacognosy: Belated Recognition of Old Friends?

By Dr. David Newman

Is the "sourcing," i.e. the identification of the plant organism(s) that actually produce the phytochemicals, now at the point that marine chemists were at approximately 25 to 30 years ago with respect to determining the actual source(s) of marine secondary metabolites? In the early 1980s, Dr. D. John Faulkner's group at the Scripps Institution of Oceanography reported the isolation and structure of renieramycin A **(1)** from a sponge collected in the Western Pacific.[1] What was unusual about this structure was that it very closely resembled a series of known antitumor agents, the saframycins A **(2)**, B and C that had been reported[2] from the terrestrial microbe *Streptomyces lavendulae* and had gone into preclinical investigations in various countries using both naturally occurring and chemically modified variations.[3] Saframycin A ultimately led to the semisynthetic production of Et743 (Yondelis(R)) many years later.

Over the next 20 years or so, it became obvious that in the marine environment, the majority of molecules found were the products of free-living microbes, with a relatively early example being the dolastatins from *Symploca* spp.[4] and a more recent one involving the demonstration of the full biosynthetic pathway of didemnin B by Xu *et al.* in 2012.[5] Perhaps the most telling example was the tour-de-force published by Piel's group[6] demonstrating the biosynthetic potential of the microbe *Candidatus Entotheonella* spp., as yet uncultivated, from the sponge *Theonella swinhoei* Y, where 31 of the 32 compounds reported from this sponge were genetically coded in the microbe.



In the plant arena, perhaps the most intriguing report in the early 1990s was the one from Stierle *et al.*[7] reporting the isolation of a fungus from the Pacific yew tree that yielded very low levels of Taxol(R) upon fermentation. Over the last 20 years, there have been reports in the literature, with one as recently as 2013, that denied that this actually occurred.[8] In this 2013 paper, the authors stated that their culture (which they claimed was the same as Dr. Stierle's but from a culture collection) and two others did not produce any taxanes, nor did they contain the gene clusters necessary for production. In contrast, the following recent papers should be consulted for the results demonstrating production of Taxol(R) by a variety of endophytic fungi, including identification of the relevant genetic machinery in the fungi investigated.[9-11] These papers demonstrate this potential, and the Soliman and Raizada paper in 2013 is of significant interest because it points out that the experiments utilized in all previous work relied upon axenic culture methods, whereas in the plant there would be significant interaction and competition between different microbes. They demonstrated increased yields when competitive fungi and other agents were introduced into the cultures, a phenomenon known to "induce" expression of cryptic gene clusters.[12] One excellent example of this type of response is the report where suspension cells of *Taxus chinensis* var. *mairei* were co-cultured in a bioreactor with its endophytic microbe, *Fusarium mairei*; a doubling of the yield of Taxol(R) was observed.[13]

Perhaps the final comment on this particular agent would be the story around the production of Taxol(R) by the hazelnut tree, first reported in 2000.[14] Quite recently, much more information plus transcriptome analyses were published by Ma et al. in 2013,[15] demonstrating the genes necessary for Taxol(R) biosynthesis. Yang et al. identified paclitaxel production in an endophyte, *Penicillium aurantiogriseum*, from hazel and identified the gene clusters involved, demonstrating evolution of the biosynthetic machinery in this *Penicillium* species independent of the plant host.[16] In this case, there is little doubt that the fungus produces the compound.

Another very well-known "plant-sourced agent" is maytansine. The trials and tribulations relating to the source(s) of this agent

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have been well documented through late 2011 by Yu et al.[17] and the consensus was that ansamitocin P3 was probably transformed within the plant to maytansine by transesterification. Recently however, Wings and coworkers reported growing axenic cultures of *P. verrucosa* but could not amplify genes involved in maytansine biosynthesis; a maytansine-producing eubacterium also could not be cultured outside of its natural habitat.[18] By using molecular techniques such as rDNA sequencing and single strand conformation polymorphism, they identified that the *Actinosynnema pretiosum* ssp. *auranticum eubacterium* present in the rhizosphere of the plant is involved in maytansine biosynthesis. A later report in 2014 confirmed the rhizosphere site for maytansine production,[19] but the exact microbe or microbial consortium was not yet identifiable. Whether the organisms are epiphytic or endophytic is not yet fully elucidated, but it does remove the "plant from contention."

There is other recent work on other "plant-sourced compounds;" an interesting report details that kaempferol **(3)** was produced by fermentation of endophytic fungi isolated from sterilized rhizomes of the high altitude plant *Sinopodophyllum hexandrum* collected in the Taibai mountains of China.[20] These scientists isolated a fungus that only produced kaempferol and then another (identified as *Mucor fragilis*) that produced both podophyllotoxin and kaempferol. Whether the first fungus had a cryptic cluster associated with podophyllotoxin was not explored.

Another recent report demonstrated that the well-known compound rohitukine's **(4)** initial sources were *Amoora rohituka* and *Dysoxylum binectariferum*. Rohitukine was later reported from *Schumanniophyton magnificum* and *S. problematicum*. Due to the therapeutic potential observed for rohitukine derivatives, such as the clinical candidates flavopiridol **(5)** and the Piramal Healthcare Ltd. compound P276-00 **(6)**, there was a search for other producers, including endophytes.

In 2012, Kumara et al. reported the production of rohitukine by fermentation of the endophytic fungus *Fusarium proliferatum* isolated from *D. binectariferum*.[21] In 2014, the same group reported that four other fungal species, three *Fusarium* isolates from *D. binectariferum* and *Gibberella fujikuroi* isolated from *A. rohituka*, also produced the compound upon fermentation.[22] They did make the point that the yield dropped during extended cultivation, though this may be due to the loss of as yet unknown co-factors.

There are other recent reports of production of compounds such as huperzines, swainsonine, and older reports such as the ergot alkaloids, that are linked to microbes isolated from plants. However, the above recent examples should be enough to start people considering the possibility that in the next few years, using and learning from the techniques pioneered in genetic analyses of marine organisms and microbes with respect to secondary metabolites, might change ideas about the actual sources of "plant-derived secondary metabolites."

To close this relatively short commentary, I will make three points that may give "food for further thought:"

- A frequent comment by phytochemical researchers is that terpene synthases are plant (sometimes fungal) biosynthetic processes. A very recent paper from the Kitasato Institute demonstrated that terpene synthases are well distributed in the actinobacteria, with over 260 being identified from total genomic analyses and some being expressed in heterologous hosts.[23]
- Chalcone synthases (and chalcones are usually considered to be plant metabolites) were identified in *Streptomyces maritimus* by Moore et al. back in 2002, as a new Type III PKS system.[24]
- In Traditional Chinese Medicine (TCM) there are very specific instructions if one goes into the literature (1,500 years plus ago), that specify site, meteorological conditions, time of year, and specific parts of a plant for collection and medicinal use.

In addition, it has often been observed and reported that general plant collections in Africa and Asia do not always yield the same active agents when recollected at or very close to the original site, but little emphasis was placed upon specific timing of recollections.

Thus, might these requirements in TCM, and recollections with observed differences, be due to the possibility that under different conditions, the microbial flora in, on, and around the plant (thinking of the plant as a host in this instance), may alter and influence the production of the desired compound(s)? Certainly a topic for continued debate. ■

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