Weed management based on natural products

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Introduction

Natural products have been important in the management of pests, used either directly as crude preparations or as pure compounds, or as resistance factors of crops. They have been often used as structural leads for the discovery and development of natural product-based pesticides. There are more examples of natural product use as fungicides, insecticides, and other pesticides than as herbicides (Pachlatko, 1998). However, there are some success stories with herbicides, and natural products remain part of the herbicide discovery strategy for those companies that still have a herbicide discovery program. Relatively little is being done to utilize natural products for weed management through allelopathy.

This review will discuss research strategies and the most important successes. Some interesting examples of natural products that have not made it to the market will also be provided, along with the reasons that some of these products were unsuccessful. Several recent general reviews of natural products as potential herbicides are available (Dayan et al., 1999a; Duke et al., 1998, 2000a, b, c; Hoagland, 2001; Hoagland and Cutler, 2000), as well as a review on the manipulation of allelopathy for weed management (Scheffler et al., 2001).

Natural products as herbicides and herbicide leads. There are several unrelated aspects of the rationale for using natural products in a herbicide discovery strategy. Nature is full of bioactive materials and compounds with unexploited properties. Many of the hundreds of thousands of secondary products generated by plants, microbes, and animals are the result of co-evolution of the producing organism with pests. Thus, the compounds have biological activity. Sometimes, the function of the compound in nature is as a phytotoxin, as with phytotoxins produced by plant pathogens or allelochemicals produced by allelopathic plants. However, very often biocides with a specific function in nature can be used for quite different purposes, as with many natural product-based pharmaceuticals. In short, biological activity is more certain with secondary compounds from nature than with randomly synthesized compounds.

Improved instrumentation has considerably reduced the cost of isolation and identification of natural compounds from what it was a decade ago. This has caused renewed interest in natural products in herbicide discovery programs. Another major reason for interest in natural phytotoxins is that they often have novel sites of action (Duke et al., 2000a, b). Even if the phytotoxin is unsuitable for commercial use, identification of a new molecular target site can be very valuable in the design of synthetic herbicides. Natural compounds or preparations may require less regulatory scrutiny for registration than synthetic compounds, thus, reducing the cost of commercializing the product. In some market niches, such as that of the home gardener, the claim that a pesticide is “natural” will appeal to the consumer. In other markets, synthetic herbicides are either not allowed (organic gardening) or are unlikely to have long-term approval (e.g., certain aquaculture situations). Lastly, despite the relatively low priority of natural products in herbicide discovery, there have been some major successes with natural products as herbicides or herbicide leads (described below). Successes lead to more interest.

Crude extracts. Crude extracts of plants or waste products of processing food have been found to effectively manage weeds in some cases. Examples are hydrolyzed maize gluten (Liu and Christians, 1997), crambe (Crambe abyssinica) waste meal (Vaughn and Berhow, 1998), and olive oil mill waste water (Aliotta et al., 2000). The biggest problems with these products are that they are not as effective as commercial herbicides and that there would never be sufficient quantity for wide-spread use. Therefore, use of a synthesized natural

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compound or natural compound derivative is a more attractive choice for major impact as a weed management tool. These crude extracts are best used in niche markets that put a premium on not using synthetic pesticides.

**Discovery strategies for natural product-based herbicides.** Hundreds of thousands of natural compounds have been isolated and their structures elucidated, but relatively few of these have been adequately tested for phytotoxicity. Rather than randomly bioassaying known compounds, structural and activity clues can be used to maximize chances of finding phytotoxic compounds.

We know that certain chemical structures are more likely to affect a particular molecular target site than others. Thus, compounds with structures that are similar to the structures of known phytotoxins or inhibitors of particular plant enzymes or functions may have similar activities. For example, usnic acid, a secondary product from some lichens, was observed to have a structure similar to that of herbicidal inhibitors of hydroxyphenylpyruvate dioxygenase (HPPD) of the triketone class such as sulcotrione. This led Romagni et al. (2000) to discover that (-)usnic acid is a better in vitro inhibitor of HPPD than the commercial herbicide sulcotrione.

Natural products that are structural analogues of metabolic intermediates or enzyme co-factors might well be inhibitors of enzymes that use those intermediates or co-factors. For example, AAL-toxin is an analogue of the sphingoid base substrates of ceramide synthase, and it is a potent inhibitor of this enzyme (Abbas et al., 1996). Another example is the sugar analogue, 2,5-anhydro-D-glucitol. It is phytotoxic (Tanaka et al., 1996) due to its structural similarity to fructose (Dayan et al., 2002). This protoxin must be phosphorylated by the enzymes hexokinase and phosphofructokinase, yielding the biphosphorylated analogue of fructose-1,6-bisphosphate. Once bioactivated, this phosphorylated sugar analog inhibits fructose-1,6-bisphosphate aldolase.

Many natural compounds have been tested for some type of biological activity other than herbicidal activity. If the mode of action of the activity is known, this information can be used to predict phytotoxicity and mode of action as a herbicide.

For example, actinonin and related compounds were known to be a potent new class of antibiotics with a unique mode of action, inhibition of peptide deformylase (Chen et al., 2000). This led Dirk et al. (2001) to find that their phytotoxicity is due to the same mechanism.

Another example is that of the fumonisins. These compounds were first known to be toxic to animals by inhibiting ceramide synthase. Fumonisins and structurally related compounds were found to be extremely phytotoxic by the same mechanism (Abbas et al., 1996). Yet another example is that of the sesquiterpene lactone, artemisinin, from annual wormwood (Artémisia annua L.). This compound was isolated from this common plant because of ethnobotanical clues from Chinese folk medicine regarding its antimalarial properties. It is highly phytotoxic (Duke et al., 1987), but the mechanism of action of artemisinin and several active analogues is unknown (Dayan et al., 1999). At least one company considered using artemisinin as the basis for development of a new herbicide. Antimalarial compounds might be expected to be herbicidal because Plasmodium spp. have a form of plastid with similarities to those of plants (Lang-Unnasch et al., 1998). Another natural product that is toxic to both plants (Zeidler et al., 1998) and Plasmodium falciparum (Jomaa et al., 1999) is fosmidomycin, an inhibitor of synthesis of all plastid terpenes.

**Choosing a biological source for discovery.** There are millions of organisms that have been inadequately studied for the biological properties of the secondary compounds that they produce. This is especially true for soil microbes. There are estimates that less than 1% of soil microbes have been cultured and identified (Pimm et al., 1995), leaving a potentially huge repository of unknown secondary compounds to be discovered. For the pesticide industry, soil microbes have been the organisms of choice for a source of natural products with potential for herbicides. Two strategies have been utilized: 1) isolation and culture of soil microbes from exotic locations and 2) manipulation of culture conditions in order to culture previously uncultured organisms. Even when an organism can be cultured, there is no assurance that the culture conditions used will be adequate for the production of every secondary compound that the organism produces in nature.
Another strategy for phytotoxin discovery with microbes is the chemical ecology strategy. This approach is to examine only those organisms for, which there is a reason to believe that a phytotoxin is produced. The most obvious case is that of plant pathogens (Sugawara, 2000). For example, a dipeptide phytotoxin, maculosin, was found to be produced by the *Alternaria alternata* that infects spotted knapweed (*Centaurea maculosa*) (Bobylev *et al*., 1996). Many, if not most, plant pathogens produce phytotoxins to kill plant cells before they use them as a food source. The greatest difficulty of this approach has been problems in adequately culturing many plant pathogens.

A chemical ecology approach can also be taken in selecting plant species for extraction of phytotoxins. If a plant is known or suspected to be allelopathic, one should expect it to produce phytotoxic allelochemicals (Macias *et al*., 2001). There are many examples of this from the literature, although relatively few of these studies have adequately fractionated the plant for all phytotoxins. In many cases, bioassay-directed isolation was not carried out and only water-soluble compounds were isolated.

Plants produce many highly phytotoxic compounds that have no obvious role in plant-plant interactions. For example, artemisinin is produced only in the glandular trichomes of annual wormwood (Duke *et al*., 1994), yet it is highly phytotoxic, as mentioned above. Hypericin, a highly phytotoxic compound, is produced only in certain cells of St. John’s wort (*Hypericum perforatum L.*) (Duke *et al*., 2000d). Both of these compounds are toxic to the producing plant. In the case of artemisinin, the actual function of the compound in nature is unknown, whereas hypericin is an antifeedant. Plants sequester or secrete phytotoxic compounds to avoid autotoxicity (discussed in detail by Duke *et al*., 2000d). Thus, examination of plants and plant parts with specialized structures for sequestration of secondary compounds is a good strategy for finding phytotoxins.

Once a biological source is found, it must be fractionated into bioactive components and compounds by bioassay-directed isolation (Duke *et al*., 2000a, b). This process has become streamlined with microbioassays that are rapid and require very little material. A recent example of this process is outlined in Rimando *et al.* (2001).

**Examples.** *Microbial Products.* Plant pathogens produce a myriad of phytotoxins that are apparently useful weakening the plant in the infection process. Most of these compounds are not host selective, even though the producing pathogen might infect only one or a limited number of host species. However, some of them are reported to be highly active against only one species or even certain genotypes of that species. Unless the host is a huge weed problem, there is little interest in herbicides that are extremely selective.

However, the literature on host-selective toxins is suspect from a herbicide standpoint. Plant pathologists have not always adequately evaluated these compounds for herbicidal activity. For example, AAL-toxin has been claimed to be a host selective phytotoxin, affecting only certain tomato varieties, yet it is one of the most generally phytotoxic natural products evaluated for herbicidal activity (Abbas *et al*., 1996). Other somewhat host-selective phytotoxins for weeds have been proposed, but not adequately tested. These include pyrenophorol for wild oat (*Avena fatua L.*) control (Kastanias and Chrysayi-Tokousbalides, 2000) and maculosin for spotted knapweed (*Centaurea maculosa*) (Bobylev *et al*., 1996).

Tentoxin is a cyclic tetrapeptide from a plant pathogen with very good selectivity against seeds in maize and soybean (reviewed in Duke *et al*., 1996). It has a unique mode of action: inhibition of both plastid processing of certain nuclear-coded proteins and energy transfer inhibition of the ATPase involved in photophosphorylation. Despite great interest, this compound was not commercialized because it was too expensive. Efforts to simply the molecule to reduce the cost of synthesis resulted in unacceptable losses in activity. This is one of many cases in which nature may have optimized the molecule.

Some of the more potent natural phytotoxins have come from non-pathogenic microbes. The bacterial phytotoxins of this type are reviewed by Barazani and Friedman (2001). A few examples, with emphasis on those that have been most successful or spurred the most interest, will be provided.
The actinomycete-produced compound, actinonin (also known as butanediamide), is a potent inhibitor of peptide deformylase (Chen et al., 2000), an important enzyme of bacteria and chloroplasts (Dirk et al., 2001). Peptide deformylase is necessary for the post-translational processing of some chloroplast genome-encoded proteins. This is a site of action unique to plants and bacteria that has potential for the development of a new herbicide class. Although it is not extremely active on whole plants, it is highly active (IC₅₀ < 100 nM) on one of two chloroplast peptide deformylases (Dirk et al., 2001).

Bialaphos and phosphinothricin, both *Streptomyces* spp. products, are successful herbicides (Lydon and Duke, 1999). Bialaphos is manufactured as a fermentation product and sold in a limited market in Japan. It is a proherbicide, requiring metabolic conversion to phosphinothricin in the target plant for herbicidal activity. Phosphinothricin is synthetically produced as glufosinate. It is the only herbicide that inhibits glutamine synthetase. It is non-selective and many crops have been engineered to be resistant to it by inserting a transgene that encodes a detoxification gene from the producing *Streptomyces*. Phosphinothricin (glufosinate) is the biggest success story for a natural product-based herbicide. It is relatively inexpensive, toxicologically and environmentally safe, and efficacious on a wide range of target weeds. Thus, with herbicide-resistant crops, it has many of the same advantages of glyphosate in glyphosate-resistant crops. This is the only example of a natural product and the gene for resistance both being used. Some companies are searching for another natural non-selective herbicide from an organism that also provides the resistance gene.

Hydantocidin is a *Streptomyces hygroscopicus* product with good herbicidal activity (reviewed by Duke et al., 2000b). It is a proherbicide that must be phosphorylated by the target plant to be an inhibitor of adeynlosuccinate synthetase. Many analogues have been patented, but none of them have been marketed. Its nucleic acid synthesis site of action may be cause for toxicological concern.

Pyridazocidin is another *Streptomyces* sp. phytotoxin with an interesting mode of action (Gerwick et al. 1997). It is only a weak phytotoxin, but it is the only natural phytotoxin known to act by the same mechanism as paraquat; by accepting electrons from photosystem I and transferring them to molecular oxygen to generate superoxide radical.

Cornexistin and hydrocornexistin are products of a non-pathogenic fungus *Paecilomyces variotii*. Both of these compounds are very phytotoxic and have been patented as herbicides (Fields et al. 1996). Cornexistin has a unique molecular target site, aspartate amino transferase (Amagasa et al., 1994). The two compounds have different selectivity on crops and weeds.

We have mentioned the lichen product, usnic acid, above. Other lichen metabolites, such as lecanorin and gyrophoric acid (Roja et al., 2000), are also phytotoxic and act by inhibiting photosynthetic electron flow by binding to the Q₈ binding site of photosystem II.

New and interesting phytotoxins from microbes are being discovered at a high rate. For example, phomalactone was recently found to cause rapid electrolyte leakage of plant plasma membranes, leading to plant death (Fukushima et al., 1998). Resormycin (Igarashi et al., 1997) and the nigrosporins (Tanaka et al., 1997) are interesting new phytotoxins with activity against both grasses and broadleaf weeds.

*Phytochemicals.* Plants produce a very large number of interesting phytotoxins with potential for use as herbicides. For example, Leptospermone is an allelochemical from which the triketone class of herbicides was developed (Mitchell et al., 2001). This is perhaps the most successful development of a commercial herbicide from a phytochemical.

Sorgoleone is an allelochemical exuded from in oily droplets from the roots of sorghum (*Sorghum bicolor*). The concentration of sorgoleone in soils growing sorghum can reach 10⁻⁴ to 10⁻⁵ M (Netzly et al., 1988) that leads to the suppression of the growth of weeds (Forney and Foy, 1985). This potent phytotoxin represses the growth of large crabgrass seedlings (*Digitaria sanguinalis*) with a GR₅₀ of 10 µM for shoot and root growth.
(Nimbal et al., 1996). Inhibition of shoot and root growth of velvetleaf (Abutilon theophrasti) and barnyardgrass (Echinochloa crus-galli) also have been observed at higher concentrations ranging from 10 to 200 µM. Sorgoleone inhibits photosynthetic electron transport in thylakoids by competing for the plastquinone binding site on photosystem II. Its activity at the molecular level is comparable to synthetic herbicides with the same site of action (Nimbal et al., 1996; Gonzalez et al., 1997).

Space does not permit providing details on the hundreds of other phytochemicals with reported herbicidal activity.

Allelopathy. Allelopathy of crops and cover crops has been studied as a tool in the management of weeds. Allelopathy is analogous to host plant resistance to insects and pathogens. It has the potential to be extremely cost effective and environmentally superior to synthetic pesticides. However, it has not been a mainstay of weed management, even before the advent of synthetic herbicides.

Allelopathic cover crops can be used to greatly reduce herbicide use. However, in agronomic crops, growing a cover crop for weed management is not economically competitive with most modern weed management systems. A highly allelopathic crop is more desirable using a cover crop. Despite, breeding efforts (e.g., Putnam and Duke, 1974; Olofsdotter et al., 1996), no commercial variety of a crop is sold for its weed-fighting capabilities. Allelopathy might be improved sufficiently by genetic engineering for it to be clearly useful tool in weed management (Scheffler et al., 2001). Work is underway to manipulate both the hydroxamic acid pathway in small grains and sorgoleone biosynthesis in Sorghum spp. This work is in its infancy, and there are many potential pitfalls; however, success with this approach could profoundly influence weed management.

**Literature cited**


