Screening for Natural Product Herbicides

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Natural products have long been used to manage pests, particularly as insecticides and fungicides. However, their usefulness as herbicides has been limited. Only one commercial herbicide is a natural product and a handful of others are natural product-like. However, the continuing emergence of herbicide resistant weeds has renewed the interest for new herbicide chemical classes with new potential molecular target sites. There are a number of advantages in utilizing natural products for the discovery of new herbicides, but there are also a number of problems or limitations associated with using such compounds (Table 1).

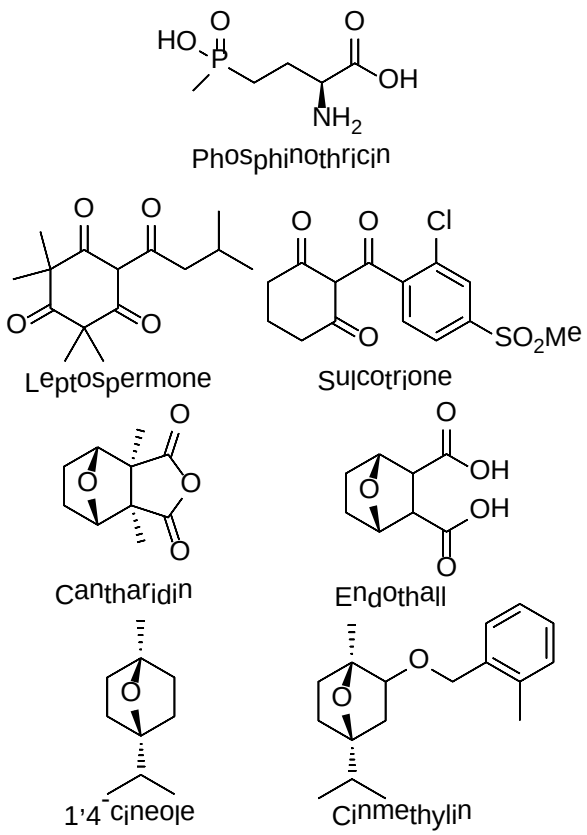
Table 1. Advantages and limitations of using natural products as a source of new herbicides or new modes of action.

Advantages	**Limitations**
New structural backbones extending to unexplored chemical spaces	Complicated structures that may be too expensive to synthesize
New molecular target sites	May have high general toxicity problems
Evolved biological activity increase the likelihood of discovering relevant structures	Structure may already be optimized for activity but have inadequate physicochemical properties
Improved instrumentation makes identification easier and requires smaller amounts	Rediscovery of known compounds is costly and sourcing may be limiting
Generally environmentally friendly	Too short environmental half-life
Better public acceptance	Public expects low rate use
May be cheaper to register	Patent protection may be limited

Investigating natural products as herbicides is advantageous because many secondary metabolites have been selected over time to address specific biological stresses. Therefore, it is likely to lead to the discovery of biologically active compounds that often have new target sites. Additionally, natural products tend to have unique scaffolds that are rich in oxygen and nitrogen molecules, and possess more chiral centers than synthetic pesticides. Such structures explore chemical spaces not exploited by their synthetic counterparts. These features, however, can sometimes be a problem because many natural product target sites may be unsuitable for a herbicidal mode of action due to general toxicity.

Determination of the mode of action of phytotoxins is a challenging endeavor due to the multitude of potential molecular targets. This short review will describe commercial herbicides that are either natural products or natural products-derived, and approaches to screening natural products for herbicides.

Bialaphos, a tripeptide analog of phosphinothricin (Figure 1), is the only natural broad-spectrum post-emergence herbicide (Figure 1). This fermentation product from *Streptomyces hygroscopis* cultures is marketed as a herbicide in eastern Asia. Bialaphos is a proherbicide that is bioactivated into phosphinothricin by plants before exerting its herbicidal action as a glutamine synthetase inhibitor. There are no other commercial herbicide with this mode of action. The commercial version of phosphinothricin is commercialized as glufosinate.



The triketone herbicides were derived from leptospermone (Figure 1), a herbicidal natural triketone component produced by bottlebrush (*Calistemon* spp.). Triketone herbicides inhibit *p*-hydroxyphenylpyruvate dioxygenase (HPPD), disrupting biosynthesis of carotenoids and causing bleaching (loss of chlorophyll).

Endothall (Figure 1) is a natural product-like herbicide that resembles cantharidin, a toxin produced by the blister beetle (*Epicauta* spp.). Endothall and cantharidin are strong inhibitors of plant serine/threonine protein phosphatases. This herbicidal mechanism of action is unique to endothall.

Figure 1. Phosphinothricin (the only natural product herbicide) and the similarity between some natural products (left) and their structurally related commercial herbicides (right).

Cinmethylin (Figure 1) is a structural analog of 1,4-cineole, a monoterpene present in the essential oils of many aromatic plants. The benzyl ether moiety was added to the monoterpene to lower the volatility of the natural product. A physionomics investigation of the mode of action of cinmethylin discovered a novel mechanism of action for herbicides, namely inhibition of plant tyrosine aminotransferase.

Screening for Natural Product for Herbicide Discovery

The successful examples mentioned above provide a good rationale for screening natural products to discover new herbicides. Investigating compounds from exotic organisms is a fairly common strategy. Phytotoxins from microbial origin are particularly interesting because large scale fermentation enables the production of sufficient amounts of toxins for agricultural use. Rediscovery of compounds is fairly common but the process is much faster with newer

dereplication processes integrating analytical instrumentation and informatics. New interfaces between HPLC, mass spectrometry (MS) and nuclear magnetic resonance simplify the isolation and identification of natural products. Commercial, public and private databases of natural products as also available to identify previously known compounds.

The outcome of the isolation process is dependent on the sorts of bioassays used. These can range from enzymatic assays to whole organism assays. In general, target site-specific assays can be automated and miniaturized for high-throughput screenings but are likely to miss a large number of potential herbicidal compounds. We prefer miniaturized whole organism bioassays. These are slower but may be more suitable for natural product-based discovery processes. Indeed, bioassay-guided fractionation protocol based on *in vivo* responses minimizes the risk of missing active compounds (that would be overlooked in site-specific assays), and maximizes the possibility of discovering new molecular sites of action.

Carefully planned dose-response experiments that use whole organisms can yield important qualitative and quantitative information in evaluating the effect of the inhibitor, and also may offer some hints as to the possible sites targeted by the compound. We currently use the free statistical software R with the DRC module developed by Streibig and Ritz in Denmark. This program easily calculates the concentration necessary for any level of inhibition as well as calculating the selectivity index.

A great number of natural products with interesting phytotoxic profiles have been discovered but very few have been studied to the extent necessary to be considered as candidate compounds. Table 2 summarizes some of the better natural products to have been considered as herbicides.

Table 2. Relevant information on the natural products mentioned in the text.

Compound	Mode of action	Unique	Patent for herbicide use
Microbial source			
Thaxtomin A	Cellulose synthesis	New	Yes
Cyperin	Enoyl-ACP Reductase	New	No
Actinonin	Peptide deformylase	New	Yes
Phaseolotoxin	Ornithine carbamoyl transferase	New	No
Hydantocidin	Adenylosuccinate synthetase	New	Yes
Albucidin	Adenylosuccinate synthetase	New	No
Tentoxin	CF1 ATPase	New	No
Pyridazocidin	Photosystem I electron acceptors	No	No
Cinnacidin	Jasmonic acid-mimic	New	No
Ascaulitoxin	Unknown	New	No
Plant source			
Pelargonic acid	Removal of cuticles	New	Yes
Sarmentine	Removal of cuticles	New	Yes
Citral	Microtubule polymerization	New	Yes

Thaxtomin A (Table 2) is a phytotoxic cyclic dipeptide analog produced by *Streptomyces scabies* and other *Streptomyces* species, the causative agents of common scab disease in potato and other taproot crops. Thaxtomin inhibits cellulose synthesis by affecting the formation of the

cellulose synthase complexes on the outside of the plasma membrane. This mode of action is different from that of known cellulose biosynthesis inhibiting herbicides such as dichlobenil and isoxaben, though the symptoms of the plants are similar.

Cyperin (Table 2) is produced by several fungal plant pathogens. This phytotoxic natural diphenyl ether that causes light-independent membrane degradation. We recently discovered that cyperin inhibits enoyl (acyl carrier protein) reductase (ENR). ENR is the molecular target site of the diphenyl ether triclosan which is commonly used as a component of antimicrobial soaps, but this enzyme has not been targeted by any commercial herbicide to date.

Actinonin (Table 2) is a naturally occurring hydroxamic acid pseudopeptide produced by a soil actinomycetes. It inhibits metallopeptidase peptide deformylase involved in initiating protein translation in prokaryotes by removing the *N*-formyl group from *N*-formyl methionine. Actinonin effectively controls a wide range of plants, including many agriculturally important and difficult-to-control weed species. This compound has been patented for herbicide use but no commercial product has been developed to date.

Phaseolotoxin (Table 2) is a sulfodiaminophosphinyl peptide produced by *Pseudomonas syringae* pathovars, the causal agent of halo blight on legumes. It is a competitive inhibitor of ornithine carbamoyl transferase.[51] Ornithine carbamoyl transferase is a key enzyme in the urea cycle which converts ornithine and carbamoyl phosphate to citrulline. No commercial herbicides have been developed to target this enzyme.

Hydantocidin (Table 2) is produced by different *Streptomyces* strains and has been the subject of intense research. It was at one time seriously considered as a natural herbicide,[52,53] but the cost of synthesis appeared prohibitive. Hydantocidin is a proherbicide that must convey bioactivity via phosphorylation in order to inhibit adenylosuccinate synthetase, and enzyme involved in purine biosynthesis.[54] The toxicological implications of this molecular target site may also have deterred development of a herbicide with this target site.

Albucidin (Table 2) was isolated from *Streptomyces albus*. The compound is a very potent nucleoside toxin that induces chlorosis and bleaching. Albucidin has moderate levels of pre-emergence activity, with broadleaf weeds being more sensitive than grasses. Pre-emergence herbicidal activity implied that the mechanism of action may involve metabolic perturbation not limited to bleaching, as the development of the majority of affected plants was halted at the cotyledonary stage. Post-emergence activity was broad spectrum. .

Tentoxin (Table 2) is a cyclic tetrapeptide produced by *Alternaria alternata* that causes extreme chlorosis of the foliage of sensitive species by inhibiting chloroplast development. Tentoxin inhibits the energy transfer of the chloroplast-localized CF1 ATPase. Tentoxin also interferes with the transport of the nuclear-coded enzyme polyphenol oxidase into the plastid of sensitive plants, but does not affect the transport insensitive species. The linked relationship between the effect of tentoxin on the β subunit of proton ATPase and polyphenol oxidase processing is not understood.

Pyridazocidin (Table 2) was purified from cultures of *Streptomyces*. Post-emergence application of pyridazocidin produced necrosis at high concentration and chlorosis at lower application rates. Pyridazocidin is positively charged and appears to act like bipyridinium herbicides (e.g. diquat) but disrupting photosystem I electron transport, resulting in rapid membrane lipid peroxidation.

Cinnacidin (Table 2) was isolated from a fungal fermentation extract of *Nectria* sp., a plant pathogen that causes cankers on many tree species. Cinnacidin causes stunting and chlorosis that spread throughout the foliar tissues. Its mode of action may be similar to that of coronatine and acts as a hormone-like herbicide by mimicking the role of jasmonic acid.

Ascaulitoxin has been isolated from the plant pathogen *Ascochyta caulina*. This natural product is already patented as a mycoherbicide. Its activity is associated with the production of the phytotoxin ascaulitoxin and its non-protein amino acid aglycone (2,4,7-triamino-5-hydroxyoctanoic acid) (Table 2). The mode of action is unknown but appears to be novel, possibly involving amino acid amino acid transporters.

Sarmentine (Table 2) is an example of the ethnobotanical approach to herbicide discovery from natural products. The fruits of long pepper (*Piper longum* L.) have been used in traditional medicine for the treatment of several diseases and ailments. Therefore, it is likely that this plant possesses a number of bioactive compounds. The bioassay-guided purification of the crude extract of long pepper led to isolation of the broad-spectrum contact natural herbicide sarmentine. The phytotoxicity of sarmentine matched that of herbicidal fatty acids such as pelargonic acid (Table 2). These molecules are broad-spectrum, foliar-applied, post-emergent herbicides that lead to plant desiccation and burndown.

Citral (Table 2) is a diterpene component of many plant essential oils that can account for up to 80% of the steam distillate, as in lemongrass (*Cymbopogon citratus* Stapf.). Citral is patented as a herbicide and is the active ingredient of a number of lemongrass oil-based natural herbicides. Citral disrupts plant microtubule polymerization rapidly. The phenomology of citral action on microtubule is distinct from that of well known mitotic inhibitors used as herbicides, such as oryzalin, suggesting that it may have a novel target site in disrupting mitosis.

Suggested Literature

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