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# Rationale for a natural products approach to herbicide discovery

**Franck E Dayan,* Daniel K Owens and Stephen O Duke**

## Abstract

**Weeds continue to evolve resistance to all the known modes of herbicidal action, but no herbicide with a new target site has been commercialized in nearly 20 years. The so-called 'new chemistries' are simply molecules belonging to new chemical classes that have the same mechanisms of action as older herbicides (e.g. the protoporphyrinogen-oxidase-inhibiting pyrimidinedione saflufenacil or the very-long-chain fatty acid elongase targeting sulfonylisoxazoline herbicide pyroxasulfone). Therefore, the number of tools to manage weeds, and in particular those that can control herbicide-resistant weeds, is diminishing rapidly. There is an imminent need for truly innovative classes of herbicides that explore chemical spaces and interact with target sites not previously exploited by older active ingredients. This review proposes a rationale for a natural-products-centered approach to herbicide discovery that capitalizes on the structural diversity and ingenuity afforded by these biologically active compounds. The natural process of extended-throughput screening (high number of compounds tested on many potential target sites over long periods of times) that has shaped the evolution of natural products tends to generate molecules tailored to interact with specific target sites. As this review shows, there is generally little overlap between the mode of action of natural and synthetic phytotoxins, and more emphasis should be placed on applying methods that have proved beneficial to the pharmaceutical industry to solve problems in the agrochemical industry.**
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**Keywords:** natural products; microbial toxins; allelochemicals; mode of action

## 1 INTRODUCTION

The therapeutic properties of bioactive natural products have benefited humankind since prehistoric times. At the onset of the 'western medicine' era, chemists began analyzing medicinal plants and discovered numerous active ingredients (e.g. aspirin, digitoxin, morphine, quinine and artemisinin) that still have a tremendous impact on human health. Today, approximately 80% of pharmaceutical drugs have been generated from natural products and analogs derived from natural products.[1] Companies have such enthusiasm for this type of chemistry that many have adopted chemical discovery strategies to optimize the 'natural likeness' of molecules channeled in high-throughput screens and combinatorial synthesis programs.[2–8]

Natural products also have a history of use as pest management tools. Many treatises on agricultural practices published by ancient Greek and Roman scholars (e.g. Theophrastus, Cato the Censor, Vergil, Columella and Pliny the Elder) mention the application of essential oils for pest control. In the Far East, more than 200 plant species were known to have pesticidal properties during the Shengnong Ben Tsao Jing era (AD 25–220).[9] More recently, the discovery of botanical insecticidal powders from *Chrysanthemum* spp. flower heads and *Derris elliptica* root led to the identification of pyrethrum and rotenone respectively. Natural products have, however, had less of an impact on modern pest management than on medicine.[10] As of 2004, approximately 11% of global sales of agricultural pesticides are either natural products or compounds that trace their discoveries back to bioactive natural products.[11]

Natural-product-based discovery has been the least successful in the area of weed management.[12–14] There are only a handful of examples of either natural products or natural-product-like compounds used for weed management. However, as the number of cases of herbicide-resistant weeds continues to increase, there is a need for new chemical classes of herbicides. In particular, there is a pressing need for compounds with new modes of action and new molecular target sites.[15] This review will discuss the potential benefit of research programs focusing on natural products as sources of new herbicide structures and new mechanisms of herbicidal action. Focus will be placed on successful natural-product or natural-product-like herbicides, and this will be extended to include recent discoveries that validate the view that natural products may play an important role in future herbicide discovery.

## 2 ADVANTAGES AND LIMITATIONS OF NATURAL PRODUCTS

Utilizing natural products for the discovery of new herbicides offers a number of advantages, but it is far from being a panacea. There are a number of problems or limitations associated with using such compounds for large-scale weed management (Table 1). No compound has all of the limitations or advantages listed in Table 1, but the listed traits are generalizations that apply to many natural compounds.

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**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 increases 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 | Excessively short environmental half-life |
| Better public acceptance | Public expects low-rate use |
| May be cheaper to register | Patent protection may be limited |

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

| Compound | Mode of action | Patent for herbicide use | Commercialized |
|---|---|---|---|
| Microbial source | | | |
| Thaxtomin A | New | Yes[41,42] | No |
| Cyperin | New | No | No |
| Actinonin | New | Yes[49] | No |
| Phaseolotoxin | New | No | No |
| Hydantocidin | New | Yes[53,54] | No |
| Ribofuranosyl triazolone | New | No | No |
| Albucidin | New | No | No |
| Anhydro-D-glucitol | New | No | No |
| Tentoxin | New | No | No |
| Pyridazocidin | No | No | No |
| Syringomycin | No | No | No |
| Macrocidin | New | Yes[68] | No |
| Cinnacidin | New | No | No |
| Ascaulitoxin | New | No[69] | No |
| Plant source | | | |
| BOA/DIMBOA | New | Yes[75] | No |
| Pelargonic acid | New | Yes[81] | Yes |
| Sarmentine | New | Yes[79] | No |
| Citral | New | Yes[86] | Yes |

There has been a dearth of truly innovative chemical classes of herbicides in the past two decades. Including natural-product backbone structures in discovery programs ensures a source of strikingly unique scaffolds for future development. Indeed, natural products are oxygen- and nitrogen-rich molecules that possess more stereogenic centers and $sp^3$-hybridized carbons than synthetic compounds. They may also contain sulfate or phosphate groups, whereas halogenation is uncommon.[16] However, the structural complexity of many natural products may be one of the drawbacks of these molecules. The costs associated with their synthesis for agricultural use may be prohibitive, whereas this issue is less critical for the pharmaceutical industry.

The structurally elaborated architecture of natural products evolved over eons to address specific biological stresses, which favors the discovery of biologically active compounds. As these compounds explore traditionally uncharted chemical spaces in comparison with conventional synthetic molecules,[17] they are more likely to interact with new target sites, as illustrated in Table 2. This is important because the new pesticide registration guidelines in the United States no longer consider the toxicological potential of pesticides individually, but rather evaluate the risk of aggregate exposure of entire classes of pesticides with similar sites of action. Furthermore, evolution of resistance to most of the currently available herbicides makes the discovery of new sites of action a particularly pressing issue for agrochemical companies and farmers.

One of the indirect and important benefits of the chemical composition and structural characteristics of natural products (e.g. the absence of 'unnatural' ring structures and the low amount of 'heavy' atoms) is that most of these compounds are rapidly degraded in the natural environment. This accounts for the perception that most natural products are environmentally benign. However, this is possibly one of the Achilles' heels of natural products. Their physicochemical properties may be less than ideal for uptake into and translocation in plants to produce an adequate effect at an economical dose. The rate of degradation of natural products may also be too rapid to allow their development as successful herbicides.

The propensity of nature to have selected biologically active molecules is not without other limitations, as many natural-product target sites may be unsuitable for a herbicidal mode of action owing to lack of specificity. For example, the microbial phytotoxins tagetitoxin and carbocyclic coformycin inhibit nucleic acid synthesis by distinct mechanisms. This raises toxicological concerns about their suitability for weed management. Also, the mammalian toxicity of ceramide synthase inhibitors (e.g. fumonisins and AAL-toxins) is problematic for these otherwise highly phytotoxic classes of compounds.[18]

While the problem of rediscovery is still an issue, new instrumentation enables the rapid identification of natural products directly in crude extracts or from minute amounts of pure compounds. These dereplicative tools circumvent what was once the costly and time-consuming process of purifying and isolating previously known molecules.[19–21]

Finally, registration of natural products for pest management in the United States benefits from a special track that makes the registration process less expensive and more rapid under the biopesticide category. However, protection of intellectual rights of natural products may sometimes be limited, which undoubtedly is a concern for the agrochemical industry.[22] Nevertheless, if the number of natural products currently patented for drug use can be used as an indication of the pharmaceutical industry's ability to obtain such intellectual rights protection,[1] there is no reason to believe that the same would not be true for its agricultural counterpart.

## 3 NATURAL PRODUCTS AS SOURCES OF HERBICIDES

### 3.1 Bialaphos (bilanophos) and glutamine synthetase

The topic of natural-product herbicides cannot be covered without featuring the success story of phosphinothricin [2-amino-4-(methylphosphinato)butanoate] (Fig. 1). Both phosphinothricin (glufosinate) and bialaphos (a tripeptide analog) are broad-

spectrum post-emergence herbicides that can be used to control a wide range of weeds in agricultural settings.[23] Bialaphos (sometimes called bilanophos) is obtained from fermentation cultures of the actinomycete *Streptomyces hygroscopis* and is marketed as a herbicide in eastern Asia. Bialaphos is a proherbicide that is bioactivated into phosphinothricin by plants before exerting its herbicidal action. The organism also directly produces a small amount of free phosphinothricin. Glufosinate, the synthetic form of phosphinothricin, is produced as a herbicide by chemical synthesis in the rest of the world (reviewed by Duke and Dayan[24]).

Bialaphos is an especially unique inhibitor in that its phosphinothricin moiety possesses a C–P–C bond. This unusual P-methylated amino acid is a structural analogue of glutamate and acts as an inhibitor of glutamine synthetase.[25] While no other herbicide has been developed for this target site, a number of other natural products have the same target site. For example, *Pseudomonas syringae* pathovars produce tabtoxin, a tabtoxinine-$\beta$-lactam-linked dipeptide proherbicide. This natural product is bioactivated via cleavage of its peptide bond to threonine, releasing the toxin tabtoxinine-$\beta$-lactam (see the review by Duke and Dayan[24]).

Glutamine synthetase is required for the production of glutamine and, perhaps even more importantly, for ammonia detoxification. Inhibition of this enzyme results in a reduction in the cellular pool of glutamine and an increase in ammonia to toxic levels. This interrupts photosynthesis and leads to death within a few days.

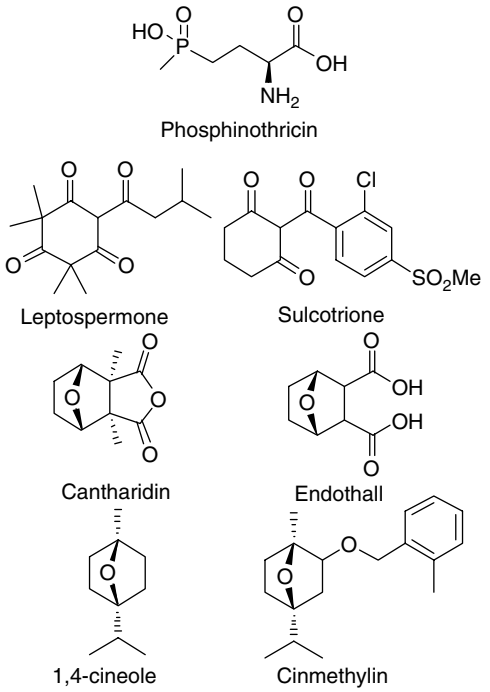


**Figure 1.** The natural herbicide phosphinothricin and the similarity between some natural products (left) and their structurally related commercial herbicides (right).

### 3.2 Triketone herbicides and *p*-hydroxyphenylpyruvate dioxygenase

The discovery of triketone herbicides is somewhat controversial because two differing accounts have been published. One story recalls scientists in California identifying the already known natural triketone leptospermone (Fig. 1) as the active component of an allelopathic plant called bottlebrush (*Calistemon* spp.). This molecule caused bleaching of plant tissues, and optimization of the triketone backbone supposedly led to the development of the commercial triketone herbicides (e.g. sulcotrione).[26,27] However, a different report suggests that the discovery may have been more serendipitous.[28] In that report, triketone herbicides were obtained from a program aiming to discover new acetyl-CoA-carboxylase-inhibiting herbicides. Regardless of their origins, the triketone herbicides are structurally similar to their phytotoxic triketone counterparts produced in nature.

Prior to the discovery of the triketone herbicides, all herbicides that caused bleaching of foliage inhibited phytoene desaturase, a key enzyme in carotenoid synthesis. However, this new chemical class had no direct effect on phytoene desaturase. A fortuitous report on the activity of similar compounds on tyrosine hydroxylase suggested that this new class of herbicide may inhibit this enzyme in plants. To test this hypothesis, the effect of triketones on tyrosine levels in rats was examined, but tyrosine hydroxylase was not inhibited. Another potential target, tyrosine aminotransferase, was subsequently eliminated as well. Continued efforts led to the identification of *p*-hydroxyphenylpyruvate dioxygenase (HPPD) as the target site of triketones in animals. This enzyme target site was afterwards validated as a new herbicide mode of action in plants.[26]

Inhibition of this enzyme disrupts the biosynthesis of carotenoids and causes bleaching (loss of chlorophyll) of the foliage similar to that observed with inhibitors of phytoene desaturase (e.g. norflurazon), but this activity occurs via a different mechanism. Inhibition of HPPD stops the synthesis of homogentisate, which is a key precursor of the tocochromanols (tocopherols and tocotrienols) and prenyl quinones. One of the prenyl quinones, plastoquinone, is an essential cofactor for phytoene desaturase activity. In the absence of plastoquinone, phytoene desaturase activity is interrupted. Bleaching of the green tissues ensues, mimicking inhibition of phytoene desaturase.[29]

The essential oil of manuka (*Leptospermum scoparium*) is rich in natural $\beta$-triketones (e.g. leptospermone, grandiflorone and flavesone). This oil also causes bleaching of plants. Leptospermone is a good inhibitor of HPPD, but grandiflorone (a minor component of the oil) is a much better HPPD inhibitor.[30] Modeling of the binding of these triketones (and additional analogs) to HPPD and evaluation of the hydrophobic contribution with HINT (hydropathic interactions) demonstrated that the substrate-binding domain of HPPD consists of a lipophilic region that favors the binding of triketones with long hydrophobic side chains over those with shorter tails.[31] A number of other natural products also inhibit HPPD.[32,33]

### 3.3 7-Oxabicycloheptane-2,3-dicarboxylic acid herbicide and protein phosphatase

Cantharidin (2,6-dimethyl-4,10-dioxatricyclo-[5.2.1.0]decane-3,5-dione) (Fig. 1) is a potent toxin produced as a natural defense terpenoid from the blister beetle (*Epicauta* spp.) and the Spanish fly (*Lytta vesicatoria*). Cantharidin and its cantharadic acid (2,3-dimethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid) analog are strong inhibitors of serine/threonine protein phosphatases (PPP) in both animal and plant systems.[34,35] Protein phosphatases in balance with their kinase counterparts control the phosphorylation status, and thereby the activity, of proteins involved in such functions as signal transduction pathways and regulation of gene expression. This control can lead to effects on a number of physiological processes.

Endothall (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid), a herbicide first commercialized in the 1950s, is a structural analogue of cantharidin. It has been used primarily for weed control in aquatic environments.[23] The WSSA herbicide handbook lists endothall's mode of action as unknown, but mentions that it causes inhibition of lipid and protein synthesis, electrolyte leakage and membrane dysfunction, affects mRNA synthesis and may act as an uncoupler. However, it is now known that endothall has the same molecular target site as cantharidin as a herbicide.[36]

As mentioned above, inhibition of plant protein phosphatases has broad consequences on plants. Cantharidin and endothall inhibit many serine/threonine protein phosphatases (up to 21 enzymes), which makes evolution of a resistant target site very unlikely.

### 3.4 Cineole herbicide

Cineoles are volatile symmetrical monoterpenes present in the essential oils of many aromatic plants (e.g. *Laurus nobilis* L., *Salvia* spp., *Eucalyptus* spp., *Xanthoxylum rhetsa* D.C. and *Artemisia* spp.). One of the major constituents of these plant essential oils is 1,8-cineole (1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane). Another naturally occurring but less abundant cineole is 1,4-cineole {1-methyl-4-(1-methylethyl)-7-oxabicyclo[2.2.1]heptane} (Fig. 1). These monoterpenes are phytotoxic.[37] The phytotoxicity of cineoles and several other monoterpenes appears to be associated with the presence of an epoxide ring.

The herbicide cinmethylin {(1R,2S,4S)-rel-1-methyl-4-(1-methylethyl)-2-[(2-methylphenyl)methoxy]-7-oxabicyclo[2.2.1]heptane} is a cineole herbicide incorporating the entire monoterpene backbone of 1,4-cineole, with the addition of a benzyl ether moiety that was added to lower the volatility of the natural product.[38] Cinmethylin was in fact developed by Shell Chemical during a biorational synthetic program to discover new insecticides that targeted the glycerol-3-phosphate shuttle. A phytotoxic dioxalane intermediate was identified. Optimization of the monocyclic structures led to rigid bicyclic structures with improved herbicidal activity. Some of the bicyclic structures possessed the basic backbone of the monoterpene cineoles found in nature.

The mode of action of cinmethylin (and 1,4-cineole) has eluded scientists for a long time,[22] but a study using physionomics and metabolomics approaches suggests that this herbicide inhibits plant tyrosine aminotransferase.[39,40] If this suggestion is verified, it would be the most recent discovery of a new commercial herbicide target site.

## 4 NATURAL PRODUCTS WITH TARGET SITES NOT CURRENTLY AFFECTED BY COMMERCIAL HERBICIDES

### 4.1 Phytotoxins of microbial origin

The success of bialaphos and glufosinate indicates that plant pathogens and soil microbes are excellent sources of new phytotoxins. There are many advantages in searching such organisms for new chemical backbones that may affect new molecular target sites. The ability to produce sufficient amounts of toxins for agricultural use by large-scale fermentation makes microbes a good system. However, there are also some limitations. Many of these phytotoxins target enzymes that may prohibit their large-scale use as herbicides because of mammalian toxicities. Readers interested in this topic are referred to a recent review article.[24]

#### 4.1.1 *Thaxtomin A and cellulose synthesis*

Thaxtomin A (Fig. 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. This herbicide has been patented as a herbicide but has yet to be commercialized.[41,42] Structure–activity studies determined that the presence of a 4-nitroindole group is necessary to maintain phytotoxicity of these metabolites.[43]

Typical phenotypic responses of plants exposed to thaxtomin A include reduced seedling growth, cell swelling and lignification of cell walls. Biochemically, thaxtomin inhibits cellulose synthesis. *Arabidopsis thaliana* seedlings treated with thaxtomin A have lower crystalline cellulose and higher contents of pectins and hemicellulose in their cell wall, relative to untreated plants. This is accompanied with an alteration of the expression of genes involved in primary and secondary cellulose synthesis, as well as genes associated with pectin metabolism and cell wall remodeling. Thaxtomin A affects the formation of the cellulose synthase complexes on the outside of the plasma membrane, leading to its dissociation from the cortical microtubule cytoskeleton.[44] This mode of action is different from that of known cellulose-biosynthesis-inhibiting herbicides such as dichlobenil and isoxaben, although the symptoms of the plants are similar.[45]

#### 4.1.2 *Cyperin and plant enoyl (acyl carrier protein) reductase*

A number of fungal plant pathogens produce cyperin, a phytotoxic natural diphenyl ether that causes light-independent membrane degradation. Diphenyl ethers have been a resourceful group of compounds used to develop commercial herbicides with three different modes of action. Some diphenyl ether herbicides inhibit porphyrin synthesis by inhibiting protoporphyrinogen oxidase; others are potent inhibitors of acetyl-CoA carboxylase; aclonifen inhibits phytoene desaturase as well as protoporphyrinogen oxidase.[46] However, cyperin has a different mechanism of action, by inhibiting enoyl (acyl carrier protein) reductase of the type-II fatty acid synthase (ENR).[47] ENR is an $NAD^+$-dependent enzyme involved in the reduction of a trans-2,3 enoyl moiety to a saturated acyl chain. Functional plant ENR is a homotetramer, and each polypeptide chain forms a single domain consisting of a seven-stranded parallel $\beta$-sheet surrounded by seven $\alpha$-helices.[48] ENR is the molecular target site of the diphenyl ether triclosan which is commonly used as a component of antimicrobial soaps, but this enzyme is not the primary target site of any commercial herbicide.

#### 4.1.3 *Actinonin and peptide deformylase*

Actinonin (Fig. 2) is a naturally occurring hydroxamic acid pseudopeptide produced by soil actinomycetes. This compound has been patented for herbicide use, but no commercial product has been developed to date.[49] It is an inhibitor of the metallopeptidase peptide deformylase.[50] Peptide deformylase is a critical enzyme because it initiates protein translation in prokaryotes by removing the *N*-formyl group from *N*-formyl methionine.

Actinonin was thought to be exclusively active on prokaryotes because cytoplasmic protein translation in eukaryotes initiates with an unformylated methionine residue. However, protein synthesis in chloroplast is like that of prokaryotes. Actinonin treatment causes a rapid decrease in D1 protein synthesis and assembly into PSII monomers, and a subsequent decline in photosynthesis, ultimately leading to stunting, bleaching and necrosis. Recent investigations have demonstrated that actinonin effectively kills a

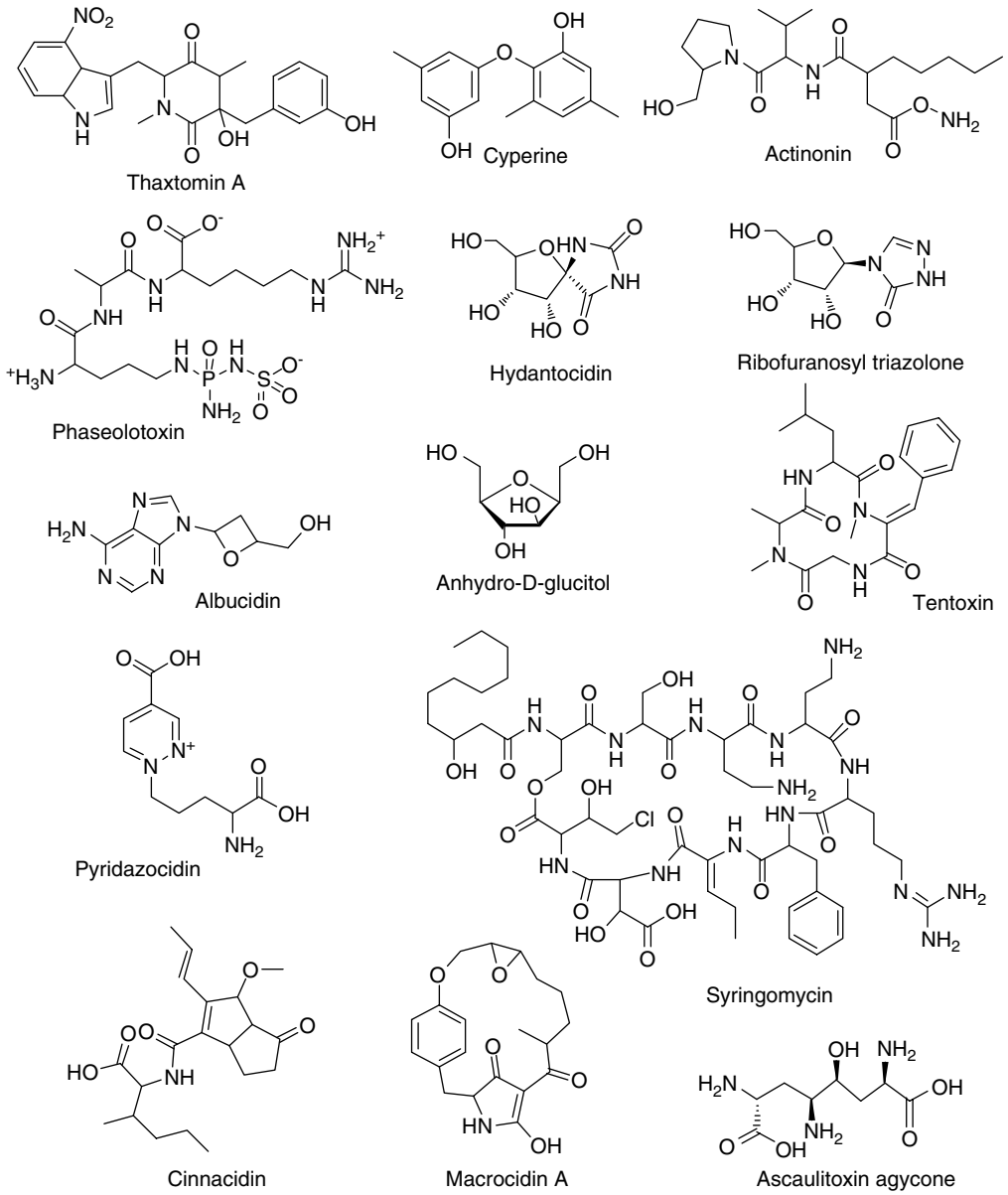


**Figure 2.** Natural products of microbial origin with target sites not currently utilized by commercial herbicides.

wide range of plants, including many agriculturally important and difficult-to-control weed species. Actinonin provides an excellent paradigm for designing and synthesizing herbicidal compounds targeting peptide deformylase, especially as overexpression of peptide deformylases leads to resistance to actinonin, which might be a useful approach to developing transgenic herbicide-resistant plants.[50,51]

#### 4.1.4 *Phaseolotoxin and ornithine transcarboxylase*

Phaseolotoxin (Fig. 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.[52] Ornithine carbamoyl transferase is a key enzyme in the urea cycle that converts ornithine and carbamoyl phosphate to citrulline. Although phaseolotoxin is a reversible inhibitor of ornithine carbamoyl transferase, it is hydrolyzed *in planta* by peptidases to produce octicidine. Octicidine, in contrast, is an irreversible inhibitor of ornithine carbamoyl transferase and the predominant form of the toxin in infected tissues. No commercial herbicides have been developed to target this enzyme.

#### 4.1.5 *Hydantocidin and adenylosuccinate synthetase*

Hydantocidin (Fig. 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,[53,54] but the cost of synthesis appeared to be prohibitive. Hydantocidin is a proherbicide that must convey bioactivity via phosphorylation in order to inhibit adenylosuccinate synthetase, an enzyme involved in purine biosynthesis.[55] The toxicological implications of this molecular target site may also have deterred herbicide development.

Ribofuranosyl triazolone (Fig. 2) is another natural phytotoxin that targets adenylosuccinate synthetase upon phosphorylation of its primary hydroxyl. However, this molecule is readily obtained

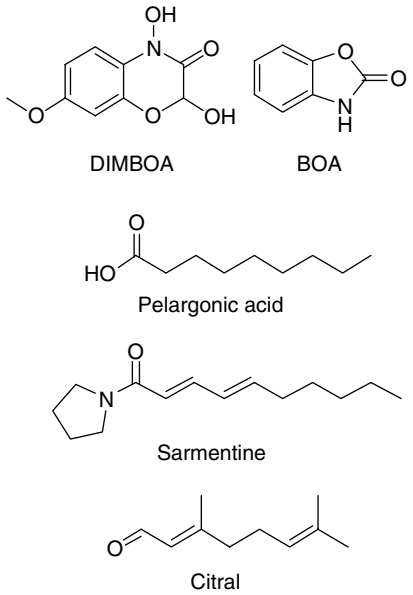


**Figure 3.** Natural products of plant origin with target sites not currently utilized by commercial herbicides.

by conventional synthetic means, which may make it a more suitable starting backbone for developing new herbicides.[56]

#### 4.1.6 *Albucidin*

Albucidin (Fig. 2) was initially discovered as a semi-synthetic derivative of oxetanocin from *Bacillus megatarium*, and was examined for its antiviral effects.[57] Subsequently, albucidin was isolated from *Streptomyces albus* subsp. *chlorinus* NRRL B-24 108 for its phytotoxic activity using a bioassay-guided fractionation.[58] The compound is a very potent nucleoside toxin, with lethality rates at less than 100 g $ha^{-1}$ in some plant species, 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. However, the onset of symptoms was extremely slow and seemed to appear preferentially in new growth tissues. From its structure, it could be hypothesized that its mode of action may be similar to that of hydantocidin. It is not known whether albucidin needs to be bioactivated prior to exerting its phytotoxic action.

#### 4.1.7 *2,5-Anhydro-D-glucitol and fructose-1,6-bisphosphate aldolase*

Bioactivation of fungal phytotoxins is not unusual. For example, 2,5-anhydro-d-glucitol (Fig. 2) is a fungal metabolite isolated from *Fusarium solani*, NRRL 18 883. This phytotoxin inhibited root growth but did not appear to have a direct *in vitro* activity.

It was noticed that it was a natural analog of fructose, and, based on the prior knowledge reported on hydantocidin (see above), it was postulated that plants would utilize this fructose analog as a substrate.[59] Indeed, 2,5-anhydro-d-glucitol was bisphosphorylated when incubated with a plant-cell-free extract in the presence of ATP. The enzymes most likely responsible for this activity are the very abundant glycolytic glucosyltransferases, hexokinase and phosphorfructokinase.[59]

Upon phosphorylation, 2,5-anhydro-d-glucitol resembles fructose-1,6-bisphosphate, but is lacking the anomeric hydroxyl group on carbon 2. This hydroxyl group is required for the formation of a covalent bond to the $\zeta$ amino functionality of a lysine residue. Binding of the phosphorylated fungal toxin to aldolase interferes with the normal catalytic function of this enzyme. However, this same phosphorylated fructose analog also inhibits mammalian aldolase,[60] which makes this particular target site questionable for a commercial herbicide.

#### 4.1.8 *Tentoxin and chloroplast ATPase*

Tentoxin (Fig. 2) is a cyclic tetrapeptide produced by *Alternaria alternata* that causes extreme chlorosis of the foliage of sensitive species by inhibiting chloroplast development.[61]

Tentoxin inhibits the energy transfer of the chloroplast-localized CF1 ATPase. Tentoxin-resistant mutants (tobacco and *Chlamydomonas reinhardtii*) carrying an amino acid substitution on CF1 ATPase suggest that this target site plays a determining role in the mode of action of tentoxin.[62] However, 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 $\beta$ subunit of proton ATPase and polyphenol oxidase processing is not understood.

#### 4.1.9 *Pyridazocidin and electron diversion from PSI*

Pyridazocidin (Fig. 2) was initially identified from cultures of *Streptomyces* sp. strain (No. 620 061) that were isolated from a soil sample collected in Honduras.[63] Upon post-emergence application, pyridazocidin led to the development of symptoms from 1–2 days after application on broadleaf and grass weeds. High application rates produced necrosis, while lower application rates induced chlorosis, with a particular impact on newly emerging leaves. The symptoms of pyridazocidin were similar to what has been observed for bipyridinium herbicides (e.g. diquat), and it was proposed that the compound had a similar mechanism of action. Bipyridinium herbicides are photosystem-I electron acceptors that reduce molecular oxygen to form a superoxide radical, which ultimately leads to the production of other reactive oxygen species that lead to rapid membrane lipid peroxidation. When isolated chloroplasts are treated with bipyridinium herbicides, the reduction of molecular oxygen to superoxide radical leads to light-dependent consumption of oxygen, known as the Mehler reaction. Pyridazocidin induced the Mehler reaction, further establishing that the compound acts through a reversible reduction/oxidation mechanism linked to photosynthetic electron transport. Pyridazocidin is the first reported natural product to manifest herbicidal activity through this type of mechanism.

#### 4.1.10 *Syringomycin and membrane perforation*

Syringomycin (Fig. 2), from *Pseudomonas syringae*, is one of the many cyclic lipodepsinonapeptide microbial phytotoxins. It is a large amphiphilic molecule with a polar peptide head and a hydrophobic 3-hydroxy fatty acid tail. This hydrophobic tail is of varying length (from C10 to C14) and is bound to the N-terminal serine residue via an amide bond. Syringomycin is composed of nine non-ribosomally synthesized amino acids bound to fatty acids. The chlorine of syringomycin is important in imparting

its biological activity.[64] Syringomycin units can assemble into macromolecules that are inserted within the cellular-membrane creating pores that are freely permeable to cations, leading to rapid necrosis in plant tissues.[65]

#### 4.1.11 Cinnacidin

The novel natural product cinnacidin (Fig. 2) was isolated from a fungal fermentation extract of *Nectria* sp. DA060097, a plant pathogen that causes cankers on many tree species. It consists of a cyclopentalenone ring system linked to an isoleucine subunit via an amide bond. Initial biological characterization of cinnacidin suggested promising herbicidal activity. Symptoms of cinnacidin include stunting and chlorosis, which spread throughout the foliar tissues.

Its mode of action may be similar to that of coronatine, although it is more active on cool-season grasses. Like coronatine, it may act as a hormone-like herbicide by mimicking the role of jasmonic acid.[66] Cinnacidin shares some structural features with jasmonic acid, and the two may have similar effects on plants, although jasmonic acid activity is transient and the activity of cinnacidin is longer lasting. The exact molecular mechanism of action remains to be elucidated.

#### 4.1.12 Macrocidins

Macrocidins (macrocidin A shown in Fig. 2) are cyclic tetramic acids that were identified in field isolates of the pathogenic fungus *Phoma macrostoma* infecting Canada thistle (*Cirsium arvense* L.). Infected tissues exhibited bleaching and chlorotic symptoms,[67] and this toxin has been patented for herbicide use.[68]

The structures of two similar macrocidins (macrocidin A and macrocidin B) were elucidated, and these compounds were subsequently tested for herbicidal activity. Post-emergence application of macrocidins caused similar chlorosis and growth inhibition on broadleaf weeds to that observed in the tissues infected by the pathogen. Grass weeds did not appear to be sensitive. Although the observed symptoms were consistent with what is typically observed for inhibitors of HPPD, *in vitro* tests against this enzyme resulted in no observable effects on enzyme activity. The mode of herbicidal action remains unknown. Bleaching and stunted growth tend to occur in new growth tissues, suggesting that the macrocidins are phloem mobile.

#### 4.1.13 Ascaulitoxin aglycone

The plant pathogen *Ascochyta caulina* has been patented as a mycoherbicide for managing weeds.[69] 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) (Fig. 2).[70]

Ascaulitoxin aglycone inhibited the growth of *Lemna paucicostata* with an $I_{50}$ lower than 1 μM. An investigation of its mode of action revealed that it acts slowly, initially stopping growth but ultimately resulting in chlorosis and death. Its activity was reversible by supplemental addition of most amino acids. Inhibition of amino acid synthesis in not the mechanism of action, because the phytotoxic effect of ascaulitoxin was also reversed by d-amino acids. A metabolomic approach revealed that the toxin caused distinct changes in amino acids, suggesting that this toxin interferes with the activity of aminotransferases. However, neither alanine aminotransferase nor alanine glyoxylate aminotransferase were inhibited by the toxin *in vitro*. Therefore, this toxin appears to have a novel mechanism of action that may involve amino acid interconversion or amino acid transporters.[71]

### 4.2 Phytotoxins of plant origin

In addition to the triketones and the cineoles mentioned in Sections 3.2 and 3.4 respectively, there are several other examples of natural products produced by plants that may serve as a source of new chemistry for the discovery of new herbicides and/or new modes of action.

#### 4.2.1 Benzoxazoline (BOA)

Benzoxazinoids are exuded in their glucosylated form by the roots of a number of grass species (e.g. wheat, rye and maize) and a few non-Poaceae species. The aglycones possess an array of biological activities including fungicidal, antimicrobial and insecticidal activity, and may also play a role in allelopathy (see Belz[72] for more information).

Some benzoxazinoids (e.g. BOA and DIMBOA) (Fig. 3) remain active in soils, and their levels correlate with the allelopathic potential for producing plant species.[73] In addition to having some soil persistence, it was demonstrated that a soil degradation product of BOA may account for most of the phytotoxicity of benzoxazinoids.[74] These compounds have been patented as herbicides, but no product has been commercialized for such use to date.[75]

The herbicidal mode of action of benzoxazinoids is still unknown, although a number of research groups have reported that these compounds interfere with both electron transport and ATPase activity in mitochondria as well as plasma membrane $H^+$-ATPase functions.[76] A recent transcriptome approach to the effect of benzoxazinoids on plants showed that these compounds had very broad effects, which made the identification of their mode of action not possible on the basis of transcriptome profiles of treated plants.[77]

#### 4.2.2 Sarmentine and fatty acids

Sarmentine (Fig. 3) 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 (reviewed by Zaveri *et al.*).[78] 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. Sarmentine has been patented as a herbicide but it is not yet commercialized.[79] The phytotoxicity of sarmentine and its analogs matched that of herbicidal fatty acids with similar tails, such as decenoic acid.[80]

Herbicidal fatty acids have a long history of use in weed control applications, and some of them have been patented and are used as natural herbicides.[81] They are broad-spectrum, foliar-applied, post-emergent herbicides that lead to plant desiccation and burndown. As with sarmentine, the herbicidal activities of fatty acids are influenced by the length of their aliphatic tails, with optimum activity against crabgrass, cucumber, velvetleaf and tobacco achieved with C9–C11 tails. The potency decreases with either shorter or longer tails.[82]

Both sarmentine and herbicidal fatty acids disrupt the plant cuticle, which leads to cell membrane damage followed by rapid desiccation and ultimate tissue death. Additionally, it has been postulated that the commercial herbicide fatty acid pelargonic acid (Fig. 3) also causes light-independent induction of cell membrane leakage, likely caused by intercalation of the fatty acid into the membrane, and a resultant light-driven peroxidase activity which

may be caused by the release of chlorophyll from its natural binding complexes.[83]

These characteristics make sarmentine and fatty acids good desiccants to aid in the harvesting of crop plants. Caprylic acid (C8) and pelargonic acid (C9) appear to be the most efficient fatty acid desiccants for use in dry bean species.[84]

#### 4.2.3 *Citral and microtubules*

Citral (Fig. 3) 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.).[85] Citral is patented as a herbicide and is the active ingredient of a number of lemongrass oil-based natural herbicides.[86,87] While high concentrations of citral are needed for adequate burndown of weeds, the vapor of citral disrupts microtubule polymerization in *Arabidopsis* seedlings within minutes of exposure.[88] The phenomology of citral action on microtubules 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.

## 5 CONCLUSIONS

This review provides but a glimpse of the structural diversity afforded by natural products. The natural process of extended-throughput screening (a high number of compounds tested on many potential target sites over long periods of time), as opposed to the human approach of high-throughput screening (a high number of compounds tested on selected target sites in very short times) enabled the selection of specifically designed compounds tailored to interact with specific target sites. In the case of herbicides, all the commercial products derived from natural products, namely phosphinothricin, the triketone herbicides, the 7-oxa-bicycloheptanes and cineoles, introduced new chemical classes of compounds with unique mechanisms of action.

Many natural products may be good phytotoxins that have modes of actions that differ from those of the current commercial herbicides (Table 2). They may be poorly suited to use as herbicides because their structures are too complex to be produced economically and the structural modifications needed to improve their physicochemical properties cause unacceptable losses of activity. However, the notable examples mentioned provide sufficient proof that natural products can also provide relatively simple structures that can be a successful approach to herbicide discovery. Interestingly, there are more patents for natural herbicides based on plant products than based on microbial toxins (Table 2), in spite of the fact that the latter are by far the more abundant.

Why the herbicide industry has not put more of its resources into semi-synthetic modification of natural products in their discovery efforts is unclear,[89] especially in light of the pressing need to introduce herbicide classes with new mechanisms of action in order to combat the evolution of herbicide resistance. One possible reason is that natural products have already been optimized for activity, and attempts to simplify their architecture often render them less active. However, this is certainly not always the case (e.g. triketone herbicides). As discussed by Duke in this issue,[90] there appears to be many factors hindering the development and introduction of new mechanisms of herbicide action, but most of these are based on economical considerations rather than on lack of scientific opportunities.[91]

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