

CALLISTO: A VERY SUCCESSFUL MAIZE HERBICIDE INSPIRED BY ALLELOCHEMISTRY

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Abstract

Mesotrione (trade name Callisto) is a new herbicide developed by Syngenta AG for control of broadleaved weeds in maize crops. Its discovery results from the study of the allelochemical leptospermone, which is produced by the roots of the "bottle brush" plant *Callistemon citrinus*. Characterisation of leptospermone confirmed its herbicidal properties but also that it was unsuitable for development as a commercial herbicide. Production of synthetic analogues using the structure of leptospermone as a lead, allowed the development of more potent compounds which included mesotrione. An overview of the development process is given and the properties of mesotrione are described.

Introduction

The search for new herbicide chemistry is an ongoing task for R&D based agrochemical companies. Lead compounds which deliver novel properties, especially new modes of action, are highly sought after and new sources of material are always being explored. The development process for new herbicides usually starts with the discovery of a relatively weak compound with interesting properties and through the production of new analogues and guided optimisation the aim is to produce a highly active compound which provides benefits to the farmer which can be successfully marketed.

The source of new lead compounds can be diverse but a field which should provide a rich source of material is that of allelochemicals. These compounds are by definition herbicidally active but may not be useful as commercial products in their natural form. They may be too complex to produce or require high rates that are not economically feasible and many have other properties which are not desirable, such as an unacceptable toxicology profile. The key is to identify relatively simple compounds that can be further optimised using synthetic chemistry to provide commercially attractive products. The development of mesotrione is an example of such a process.

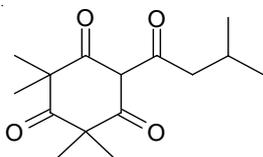
Methods

The "red bottle brush plant", *Callistemon citrinus* is a member of the Myrtaceae family. It originates from Australia but is now spread throughout the world as an ornamental plant in countries with mild climates. It is the most widely cultivated member of the genus both in Australia and overseas due to its very attractive appearance. The common name "red bottlebrush" perfectly describes the bright red flower spikes which make the plant so desirable as an ornamental.

The climate of California, USA is well suited to growing *Callistemon citrinus* and it was during 1977 that a biologist working at the California, Western Research Centre of the Stauffer chemical company (now Syngenta) noticed that very few plants were growing under the *Callistemon citrinus* bushes in his garden. The extent to which weeds were suppressed was out of proportion to what he expected from shading or leaf litter. Fascinated by this, he took samples of soil from the area immediately beneath the plants and used a series of extractions in an attempt to isolate potential allelopathic agents. The resulting five extracts were sprayed onto young *Echinochloa crus-galli* (barnyard grass) plants in the glasshouse. Activity was found in two fractions. One fraction gave relatively high activity and another gave a lower level of activity. Given the properties of the different extraction processes, this indicated that the compound responsible for the herbicidal activity was strongly acidic. A TLC plate was then run with these extracts and seeds of *E. crus-galli* were placed on the plate and germinated. The seeds germinating in one section of the plate showed clear bleaching symptoms on the emerging shoots. This band was isolated and a range of standard analytical techniques (1H NMR, IR and Mass Spec.) was used to determine the structure of the active component.

This proved to be 1-hydroxy-2-isovaleryl-4,4,6,6-tetramethyl cyclohexen-3,5-dione, (common name leptospermone) (Figure 1).

Figure 1. Chemical structure of leptospermone



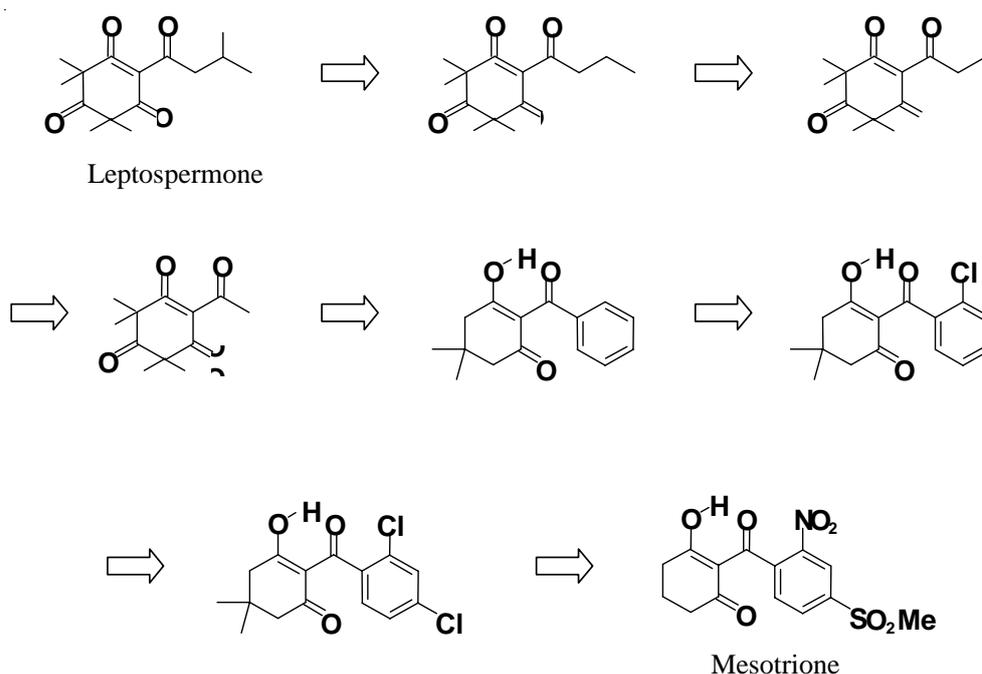
Results and further development

Literature searches revealed that leptospermone was first discovered in 1921 (Penfold 1921) and that a number of researchers had since shown that it could be extracted from a number of plants (Bick 1965, Hellyer 1966, 1968). However, it had never been associated with *Callistemon citrinus* and had never been cited as an allelochemical.

Pure leptospermone was then synthesized and tested both pre- and post emergence on a range of plant species. The species used were *Digitaria sanguinalis* (hairy crabgrass), *Setaria glauca* (yellow foxtail), *Echinochloa crus-galli* (barnyard grass), *Avena sativa* (California red oat), *Amaranthus retroflexus* (redroot pigweed), *Brassica juncea* (Indian mustard) and *Rumex crispus* (curly dock). A range of rates were tested and a rate of 9000 gai/ha (grams active ingredient per hectare) was required to give acceptable control. At this rate it was not a realistic option to develop leptospermone commercially.

The novel symptomology (bleaching of the leaves) was extremely interesting and a programme of synthesis and optimisation of analogous compounds was undertaken. The programme was directed using structure activity relationships and gradually led through to a series of more active compounds (Knudsen et al. 2000, Lee et al. 1998). Some of these compounds were extraordinarily active with weed LD90s as low as 0.001 kg/ha compared to an LD90 of 5 kg/ha for leptospermone (refined after the initial studies). Unfortunately the most active candidates were ruled out due to other characteristics such as excessive soil persistence, a lack of crop selectivity or unacceptable human toxicology. Finally, after many rounds of optimisation, and eleven years after the initial isolation of leptospermone from *Callistemon citrinus* by Syngenta chemists, the compound mesotrione was discovered (figure 2 gives a selection of a few of the thousands of compounds tested).

Figure 2. Optimisation steps from leptospermone to mesotrione



Mesotrione (2-[4-(methylsulphonyl)-2-nitrobenoyl]-1,3-cyclohexadione) is a triketone herbicide, which

gives selective weed control in maize. It is readily taken up by the leaves, shoots or the roots and is translocated in both the xylem and phloem. It can be used both pre- and post-emergence and controls a wide spectrum of broadleaved weeds and some grass weeds, which are important in maize, with excellent selectivity. It is an extremely flexible herbicide with a very wide application window and most sensitive weeds are controlled from pre-emergence applications up to at least the 6-leaf stage. The use rates of mesotrione are in the range from 75 to 225 gai/ha (around 100 times more potent than leptospermon). Table 1 gives the spectrum of weed control.

Table 1. Weed control spectrum of mesotrione

	Pre-emergence	Post-emergence		Pre-emergence	Post-emergence
Plant Names	Mesotrione 200 gai/ha	Mesotrione 100 gai/ha	Plant Names	Mesotrione 200 gai/ha	Mesotrione 100 gai/ha
<i>Abutilon theophrasti</i>	S	S	<i>Kochia scoparia</i>	S	M
<i>Amaranthus spp.</i>	S	S	<i>Lamium purpureum</i>	S	S
<i>Amaranthus rudis</i>	S	S	<i>Matricaria chamomilla</i>	S	M
<i>Ambrosia artemisiifolia</i>	S	M	<i>Mercurialis annua</i>	R	M
<i>Ambrosia trifida</i>	M	S	<i>Polygonum aviculare</i>	R	M
<i>Atriplex patula</i>	R	S	<i>Polygonum convolvulus</i>	R	M
<i>Capsella bursa-pastoris</i>	S	S	<i>Polygonum lapathifolium</i>	S	S
<i>Chenopodium spp.</i>	S	S	<i>Polygonum pensylvanicum</i>	S	S
<i>Cirsium arvense</i>	R	M	<i>Polygonum persicaria</i>	S	S
<i>Datura stramonium</i>	S	S	<i>Senecio vulgaris</i>	S	S
<i>Fumaria officinalis</i>	S	S	<i>Sinapis arvensis</i>	S	S
<i>Galinsoga parviflora</i>	S	S	<i>Solanum nigrum</i>	S	S
<i>Galium aparine</i>	R	M	<i>Stellaria media</i>	S	S
<i>Helianthus annuus L.</i>	S	S	<i>Viola arvensis</i>	S	S
<i>Ipomea hederacea</i>	M	M	<i>Xanthium strumarium L.</i>	R	S

S = Susceptible, M = Moderately Susceptible, R = Resistant

The mode of action of mesotrione has been found to be by competitive inhibition of the HPPD (hydroxy-phenyl-pyruvate dioxygenase) enzyme, which is part of the pathway that converts the amino acid tyrosine to plastoquinone. Plastoquinone is a required cofactor for the enzyme phytoene desaturase, a key enzyme in carotenoid biosynthesis. Since carotenoids are required for photosynthesis and protection of chlorophyll and plant cell membranes during photosynthesis, inhibition of this enzyme leads to plant death in sensitive species. The destruction of chlorophyll accounts for the typical white "bleaching" symptoms which are observed. This is the same mechanism of action as the original allelochemical leptospermon. Maize is naturally tolerant to mesotrione by breaking it down into inactive metabolites and it tolerates both pre-emergence and post-emergence applications with very little injury and no crop response or yield penalty (tables 2 and 3).

Table 2. Pre-emergence Crop Tolerance in Field Trials (20 trials USA)

Rate (gai/ha)	200	300	440	520
Mean Phytotoxicity	0	0	0	0

Table 3. Post-emergence Crop Tolerance in Field Trials (16 trials Europe)

Rate (gai/ha)	150	300
Mean Phytotoxicity	4	12
Yield (% untreated)	102	102

Mesotrione has a favourable toxicological and environmental profile and presents no significant risk to humans and other non-target organisms or to the environment when used as directed. It is rapidly degraded by soil microorganisms which results in the potential for mesotrione to leach to groundwater being negligible.

Mesotrione is now sold in over 30 countries worldwide and its brand name Callisto™ is derived from the Callistemon plant that inspired its discovery. It is also formulated in the pre-packaged mixtures Lumax™, Camix™ and Lexar™ for pre-emergence weed control in maize.

Since the introduction of Callisto in the United States and Europe in 2000, it has been rapidly adopted by growers. It is now the market leader for post-emergence broadleaved weed control in maize and the newly introduced pre-emergence ready-mixes are being equally quickly taken up.

Conclusion

The development and commercial success of mesotrione illustrates the potential for the use of allelochemicals as leads for the development of new herbicides. It is unlikely that many allelochemicals will have the potential to be developed as commercial herbicides per se, but in the increasingly difficult search for new chemistry and new modes of action, allelochemicals can provide the base structures for further chemical modification and the development of commercially viable herbicides.

References

- Bick IRC, Blackman AJ, Hellyer RO, Horn DHS (1965). The isolation and structure of flavesone. *Journal of the Chemical Society* 3690.
- Hellyer RO (1968). The occurrence of some beta-triketones in the steam-volatile oils of some myrtaceous Australian plants. *Aust J Chem* 21: 2825-2828.
- Helyer RO and Pinhey J (1966). *Journal of the proceedings of the Royal Society of New South Wales*, 1496.
- Knudsen CG, Lee DL, Michaelay WJ, Chin H, Nguyen NH, Rusay RJ, Cromartie TH, Gray R, Fraser T, Cartwright D (2000). Discovery of the triketone class of HPPD inhibiting herbicides and their relationship to naturally occurring beta-triketones. In S.S. Narwal et al. *Allelopathy in ecological agriculture and forestry*. Kluwer academic publishers.
- Lee DL, Knudsen CG, Michaelay WJ, Chin H, Nguyen NH, Carter CG, Cromartie TH, Lake BH, Shribbs JM, Fraser T (1988). The structure-activity relationships of the triketone class of HPPD herbicides. *Pestic Sci*, 54: 377-384.
- Penfold AR (1921). The occurrence of a new phenol in the essential oils of the "leptospermum". *Journal of the proceedings of the Royal Society of New South Wales*, 45.