Manuka Oil as a Potential Natural Herbicide

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In 1977, Gray observed that bottlebrush plant (*Callistemon citrinus*) repressed the growth of plants in its surroundings. Crude extracts from this plant caused the bleaching of grass weeds. He identified the active component as leptospermone, a natural triketone structure with no known biological activity that had been reported in a number of Australasian shrubs. Leptospermone was moderately active in greenhouse tests, controlling mostly small-seeded grass weeds. This natural product and a small number of synthetic structural analogs were patented as herbicides in 1980. A few years later, a separate group at the Western Research Center was generating analogs of the cyclohexanedione herbicide sethoxydim, an inhibitor or acetyl-coenzyme-A carboxylase. Some of the second generation herbicidal derivatives with a dimedone backbone caused bleaching symptoms similar to leptospermone. Combination of the syncarpic acid of leptospermone to this chemistry ultimately served as the basis for the development of the triketone synthetic herbicides (Fig. 1).

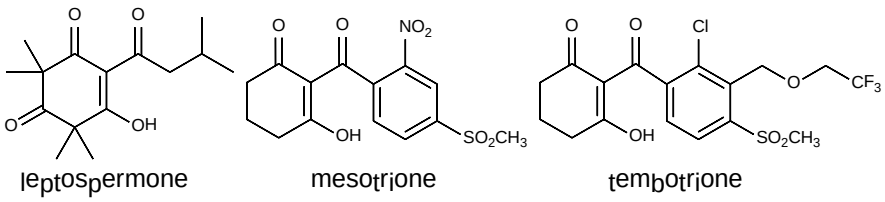


Fig. 1. Structures of the natural triketone leptospermone and two synthetic analogues that are sold as commercial herbicides.

Natural β-triketones are common in many Australasian woody plants (e.g., *Leptospermum, Eucalyptus*, *Melaceuca,* etc...). Steam distilled manuka oil account for 0.3% of the dried weight of *L. scoparium*. However, the amount of β-triketone present in these oils varies wildly across New Zealand. Some chemotypes contain as little as 0.1% triketone while others can accumulate up to 33%.

β-triketone herbicides (e.g., sulcotrione and mesotrione) cause bleaching of newly emerging tissues. This symptom was traditionally associated with inhibitors of phytoene desaturase but triketone herbicides do not inhibit this enzyme. It was later found that these herbicide inhibit *p*-hydroxyphenylpyruvate dioxygenase (HPPD), a key enzyme involved in the biosynthesis of prenyl quinones and tocopherols. Plastoquinone (a prenylquinone) is an essential cofactor for phytoene desaturase. In the absence of plastoquinone, phytoene desaturase activity is reduced which results in bleaching of young foliage and accumulation of phytoene typically observed

with phytoene desaturase inhibitors develop (Fig. 2). Chlorophyll levels are also affected because the photosynthetic apparatus is no longer protected from the reactive oxygen species generated under high light intensity.

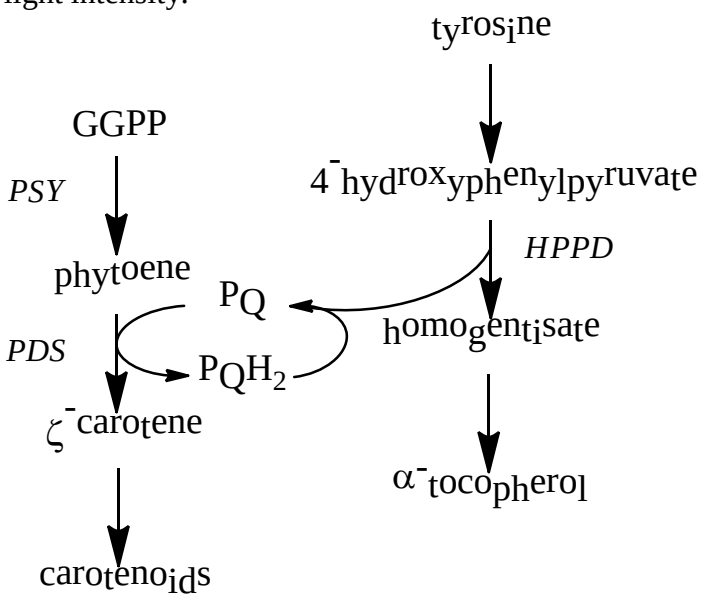
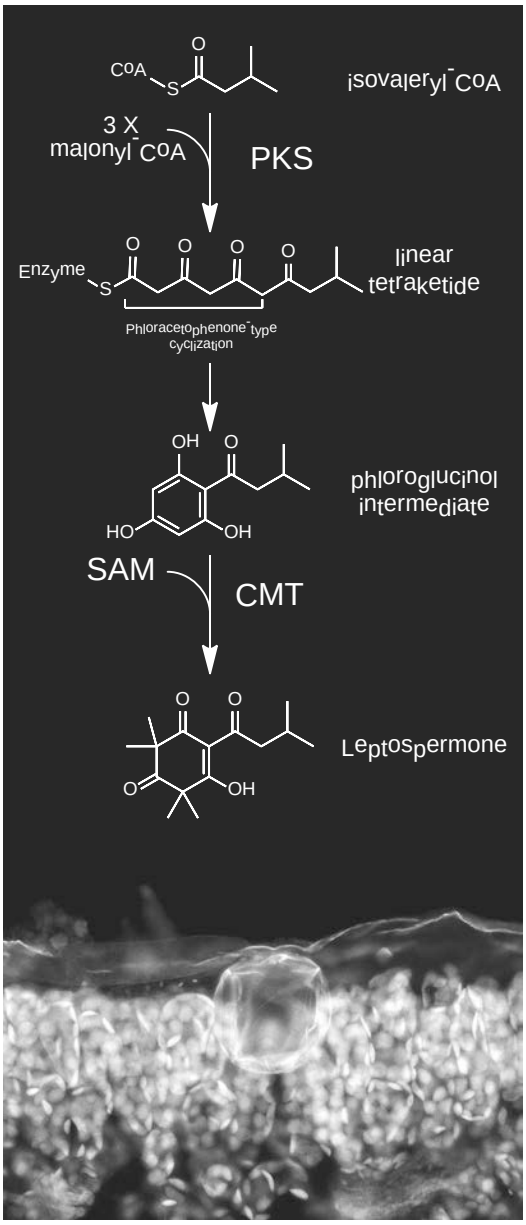


Fig. 2. Mechanism of action of leptospermone. *PSY* = phytoene synthase; *PDS* = phytoene desaturase' *HPPD* = p-hydrodyphenylpyruvate dioxygenase; PQ = plastoquinone.

Gray observed that leptospermone also caused bleaching of plant tissues. Work with the bioactive components of manuka oil demonstrated that some natural β-triketones also inhibit plant HPPD. Most of the activity of manuka oil was due to leptospermone because it was the most abundant triketone in the oil. However, grandiflorone, a minor constituent with has a more lipophilic side chain, was much more active on HPPD. Conversely, the short methyl side chain of flavesone nullified the activity. The important role of the lipophilicity of the side chain was confirmed with a structure-activity study with a series of natural and synthetic leptospermone analogs.

Manuka oil is active both when applied on the foliage and on the soil surface. While most essential oils have little to no soil activity, preemergence application of manuka oil controlled the growth of large crabgrass at a rate of 3 L/ha. The soil activity of manuka oil is due in part with the relatively slow dissipation of leptospermone, which remained active in soil for at least two weeks.

Triketones and other phytotoxic natural products are often produced and stored in specialized structures which may serve in part as a mechanism to prevent autotoxic effects. In the leaves of members of the Myrtaceae family, which encompasses most of the known herbicidal triketone-producing species, specialized schizogenous glands (Fig. 3). In the genus *Leptospermum*, the gland is covered by two to four cells which have thin, straight walls and are generally of the



same approximate size. These cells are encircled by five to 14 unspecialized epidermal cells in a spiral orientation. Schizogenous formation proceeds by the division of single cells within the epidermis or mesophyll layer with the oil cavity forming as an intracellular space. The schizogenous cavity is lined with a single layer of 4 to 6 epithelial cells that are thought to be responsible for the production of the volatile oils stored within the cavity.

Fig. 3. Proposed biosynthesis of leptospermone (top) and micrograph of a representative *Leptopermum scoparium* (manuka) schizogenous gland connected to the cuticle and extending into the mesophyll.

The chemical synthesis of natural β-triketones has been well studied, but much work remains to unravel the *in vivo* biosynthesis of these molecules. Although an *in planta* biosynthetic route has yet to be established, a hypothetical pathway can be proposed based on the structure of the final compounds (Fig. 3). In a series of conversions analogous to the well-examined chalcone synthase enzyme, a type III polyketide synthase (PKS) sequentially condenses three malonyl CoA molecules into a polyketide chain extending from an isovaleryl CoA starter molecule. The enzyme subsequently cyclizes the linear tetraketide intermediate via a Claisen type condensation to generate a phloroglucinol intermediate. A PKS enzyme, valeropenone synthase (VPS), with this activity has been purified to homogeneity and biochemically characterized from *Humulus lupulus* L. (hops) cone glandular hairs. VPS is thought to be involved in the production of the beer flavoring iso-acids of hops which have been shown to contain a β,β-triketone moiety. Subsequently, a gene for this enzyme has been identified and characterized. Efforts are currently underway to isolate and characterize enzymes homologous to VPS from *Leptospermum scoparium* as an initial effort to characterize the leptospermone biosynthetic pathway.

After the production of the phloroglucinol intermediate, the compound would be proposed to undergo spontaneous keto-enol tautomerization, and subsequently to undergo methylation by an as-of-yet unidentified C-methyltransferase (CMT). Early work with methionine-methyl-C^{14}

labeled adult *Dryopteris marginalis* ferns, demonstrated that the C- and O-methyl substituents of isolated phloroglucinols were derived from methionine. If these findings are consistent with leptospermone, the biosynthetic methyltransferases are likely to be similar to S-adenosylmethionine using CMTs identified in other species.

Suggested literature

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