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p-Hydroxyphenylpyruvate dioxygenase is a herbicidal target site for β-triketones from *Leptospermum scoparium*

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Abstract

p-Hydroxyphenylpyruvate dioxygenase (HPPD) is a key enzyme in tyrosine catabolism and is the molecular target site of β-triketone pharmacophores used to treat hypertyrosinemia in humans. In plants, HPPD is involved in the biosynthesis of prenyl quinones and tocopherols, and is the target site of β-triketone herbicides. The β-triketone-rich essential oil of manuka (*Leptospermum scoparium*), and its components leptospermone, grandiflorone and flavesone were tested for their activity in whole-plant bioassays and for their potency against HPPD. The achlorophyllous phenotype of developing plants exposed to manuka oil or its purified β-triketone components was similar to that of plants exposed to the synthetic HPPD inhibitor sulcotrione. The triketone-rich fraction and leptospermone were approximatively 10 times more active than that of the crude manuka oil, with I_{50} values of 1.45, 0.96 and 11.5 μg mL^{-1}, respectively. The effect of these samples on carotenoid levels was similar. Unlike their synthetic counterpart, steady-state O_2 consumption experiments revealed that the natural triketones were competitive reversible inhibitors of HPPD. Dose–response curves against the enzyme activity of HPPD provided apparent I_{50} values 15.0, 4.02, 3.14, 0.22 μg mL^{-1} for manuka oil, triketone-rich fraction, leptospermone and grandiflorone, respectively. Flavesone was not active. Structure–activity relationships indicate that the size and lipophilicity of the side-chain affected the potency of the compounds. Computational analysis of the catalytic domain of HPPD indicates that a lipophilic domain proximate from the Fe^{2+} favors the binding of ligands with lipophilic moieties.

Keywords: *Leptospermum scoparium*; Leptospermone; Manuka oil; Myrtaceae; Essential oil; Grandiflorone; Herbicide; *p*-Hydroxyphenylpyruvate dioxygenase; HPPD; Mode of action; Phytotoxins; Triketones

1. Introduction

p-Hydroxyphenylpyruvate dioxygenase (HPPD, EC 1.13.11.27, EC 1.14.2.2) is involved in pigment synthesis and tyrosine catabolism in most organisms. This non-heme, iron II containing, α-keto acid-dependent enzyme catalyzes a complex reaction involving the oxidative decarboxylation of the 2-oxoacid side-chain of 4-hydroxyphenylpyruvate (4-HPP), the subsequent hydroxylation of the aromatic ring, and a 1,2 (ortho) rearrangement of the

Abbreviations: HPPD, *p*-hydroxyphenylpyruvate dioxygenase; 4-HPP, 4-hydroxyphenylpyruvate; HGA, homogentisic acid; NTBC, 2-(2-nitro-4-trifluoromethylbenzoyl)cyclohexane-1,3-dione; Sulcotrione, (2-[2-chloro-4-methanesulfonylbenzoyl]-cyclohexane-1,3-dione); Mesotrione, (2-[4-methylsulfonyl-2-nitrobenzoyl]-cyclohexane-1,3-dione); Acetonitrile, MeCN; Trifluoroacetic acid, CF_3CO_2H.

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carboxymethyl group, to yield homogentisic acid (HGA) (Que and Ho, 1996; Crouch et al., 1997; Pascal et al., 1985).

HPPD is a molecular target site for pharmaceutical products in the treatment of the hypertyrosinemia, a hereditary disease that results in the unregulated degradation of tyrosine. More specifically, a deficiency in the enzyme fumarylacetoacetase causes abnormal accumulation of succinylacetoacetate, succinylacetone and 5-aminolevulinic acid, which are subsequently degraded by the liver HPPD into toxic metabolites (Lindblad et al., 1977). Inhibiting HPPD activity by β-triketone pharmacophores, such as 2-(2-nitro-4-trifluoromethylbenzoyl)cyclohexane-1,3-dione (NTBC) (**1**) (Fig. 1), reduces or prevents the formation of these harmful by-products (Lindblad et al., 1977).

HPPD is also the target site in plants of β-triketone herbicides [e.g. sulcotrione (2-[2-chloro-4-methanesulfonyl benzoyl]-cyclohexane-1,3-dione) (**2**) and mesotrione (2-[4-methylsulfonyl-2-nitrobenzoyl]-cyclohexane-1,3-dione) (**3**)] (Schulz et al., 1993; Lee et al., 1997, 1998; Pallett et al., 1998; Viviani et al., 1998). Inhibition of this enzyme disrupts the biosynthesis of carotenoids and results in bleaching (loss of chlorophyll) of the foliage of treated plants. Such phenotypic response is similar to that observed on plants treated with inhibitors of phytoene desaturase (Lee et al., 1997). However, inhibition of HPPD has a different mechanism of action. In plants, HPPD catalyzes the formation of HGA, which is a key precursor of the eight different tocochromanols (tocopherols and tocotrienols) and prenyl quinones. The latter prenylquinone is a required cofactor for phytoene desaturase (Norris et al., 1995). Therefore, inhibition of HPPD indirectly reduces phytoene desaturase activity by reducing the pool of available plastoquinone (Pallett et al., 1998). The subsequent decrease in carotenoid levels results in the destabilization of the photosynthetic apparatus. Therefore, under high light intensity, excess energy is no longer quenched, chlorophyll molecules are destroyed, and the foliage appears bleached.

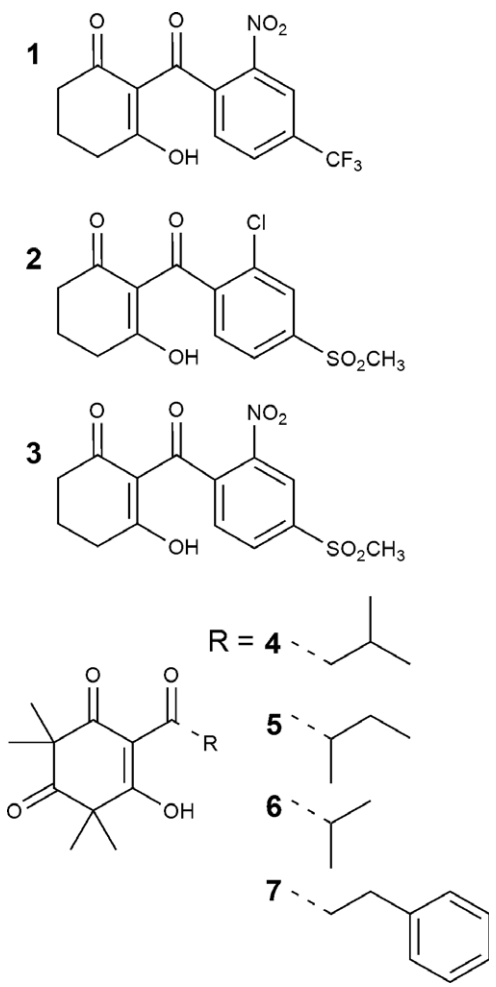

Fig. 1. Structures of the reference compounds NTBC (**1**), sulcotrione (**2**) and mesotrione (**3**), as well as the β-triketones isolated from manuka oil that were tested in this study, leptospermone (**4**), isoleptospermone (**5**), flavesone (**6**), and grandiflorone (**7**).

The triketone herbicides were apparently derived from the structural backbone of natural β-triketones following the discovery of the herbicidal properties of leptospermone (**4**) (Fig. 1) from the bottlebrush plant (*Callistemon* spp.) (Lee et al., 1997, 1998; Gray et al., 1980). The private sector has invested millions of dollars in developing and commercializing structural analogues of leptospermone (**4**) as herbicides. The mode of action of these synthetic compounds has been well studied, yet, there are no published reports on the mode of action of this natural β-triketone. The only published reports of the effect of natural products on HPPD are concerned with usnic acid (a lichen secondary metabolite), sorgoleone (a plant lipid benzoquinone) and juglone (a plant naphthoquinone) (Meazza et al., 2002; Romagni et al., 2000), which provide indirect information regarding the structural requirement for inhibition of plant HPPD by natural products.

The essential oils of several woody plants originating from New Zealand and Australia (e.g., *Leptospermum*, *Eucalyptus*, and *Callistemon* spp.) contain relatively large amounts of natural β-triketones (e.g., leptospermone (**4**), isoleptospermone (**5**), flavesone (**6**), and grandiflorone (**7**)) (Douglas et al., 2004; Hellyer, 1968). These oils and their components have antifungal, antimicrobial (Christoph et al., 2000; Spooner-Hart and Basta, 2002; van Klink et al., 2005), antiviral (Reichling et al., 2005; Spooner-Hart and Basta, 2002), and insecticidal and molluscicidal activities (Spooner-Hart and Basta, 2002). Leptospermone (**4**), one of the primary components of these oils, is also phytotoxic and causes bleaching of grass and broadleaf weeds, while maize is inherently more resistant (Gray et al., 1980; Knudsen et al., 2000).

We report here that essential oil distilled from the leaves of manuka (*Leptospermum scoparium* J.R. and G. Forst) and the β-triketone leptospermone (**4**) isolated from this oil cause bleaching symptoms very similar to those caused by synthetic triketone herbicides. The molecular target site of the natural β-triketones isolated from