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Structure–activity relationships of analogs of 3,4,5-trimethylfuran-2(5*H*)-one with germination inhibitory activities

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ABSTRACT

Smoke-derived butenolide compounds have, in recent years, been shown to be important germination signaling molecules, which also affect seedling growth. The butenolide 3,4,5-trimethylfuran-2(5*H*)-one was previously isolated from plant-derived smoke and was found to significantly reduce the effect on germination by the highly active promotor karrikinolide (KAR_1, 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one), another smoke-derived compound. In this study, 11 analogs of 3,4,5-trimethylfuran-2(5*H*)-one were synthesized and their effect on the germination of light-sensitive 'Grand Rapids' lettuce seeds (*Lactua sativa* cv. 'Grand Rapids') were evaluated. A concentration series (1 mM–1 μM) of the analogs were tested alone, or in combination with 0.01 μM KAR_1. Only two compounds were found to reduce the germination promotory effect of 0.01 μM KAR_1 in a similar manner as observed with 3,4,5-trimethylfuran-2(5*H*)-one, with activity ranging from 1 mM to 10 μM. Four compounds were found to have inhibitory activity at 1 mM and 100 μM. The retention of activity by some of the analogs may be useful for designing novel compounds with improved activity. Furthermore, understanding the structure–activity relationships of these compounds may be helpful in synthesizing molecular probes that can be used to further investigate the mechanism of action of these compounds in regulating seed germination.

Introduction

Burning vegetation produces smoke that contains highly active compounds able to promote seed germination in many species (Dixon et al., 2009; Nelson et al., 2012). The butenolide, 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one (karrikinolide; KAR_1) (**1**) has been identified as one of the most active germination compounds present in smoke (Fig. 1; Flematti et al., 2004, 2010; Nelson et al., 2012; Van Staden et al., 2004). In addition to KAR_1 (**1**), five related karrikins (KAR_2–KAR_6) have also been identified in smoke and shown to have germination promoting activity, although to lesser degrees (Flematti et al., 2009). KAR_1 improves seedling vigor and plant growth in certain instances, and karrikins have been proposed to be a new and important family of naturally occurring plant growth regulators (Chiwocha et al., 2009; Light et al., 2009). Conversely, a compound with germination inhibitory activity was also isolated from plant-derived smoke, namely 3,4,5-trimethylfuran-2(5*H*)-one (**2**) (Fig. 2; Light et al., 2010). This inhibitor (**2**) was shown to be active at 10 and 100 μM, and application together with 0.1 μM KAR_1 (**1**) suppressed germination of 'Grand Rapids' lettuce seeds in comparison to treatments with KAR_1 alone (Light et al., 2010). Similarly, in another study, **2** inhibited germination of *Arabidopsis thaliana* at 10 μM (Nelson et al., 2011).

Prior to identification of these two compounds from plant-derived smoke with opposing actions (i.e. **1** and **2**), it was well established that, in general, smoke-water is able to promote germination of many species when diluted to relatively low concentrations (Brown and van Staden, 1997; Dixon et al., 2009; Light et al., 2010). On the other hand, high concentrations of smoke-water tend to have a negative impact on germination, and it was postulated that this effect was attributable to inhibitory compounds, such as **2**, which may be present in plant-derived smoke (i.e. compounds other than promotory karrikins) (Light et al., 2010). A previous study also proposed that smoke could, therefore, possibly play a dual regulatory role during germination (Light et al., 2002), by providing the stimulus to germinate whilst temporarily preventing germination until sufficient water was available. Such a "stop-go" system could potentially have applications in agriculture or horticulture for controlling the timing of germination, particularly since the effect of **2** may be reversed by leaching the treated seeds, whereas the effect of **1** is only reversible by leaching shortly after treatment (in the case of *Lactuca sativa* cv. 'Grand Rapids') (Soós et al., 2012). Furthermore, compounds with opposing actions on germination could be useful for further understanding physiological events involved in breaking seed dormancy (Soós et al., 2012).

Abbreviation: KAR_1, karrikinolide (3-methyl-2*H*-furo[2,3-*c*]pyran-2-one).

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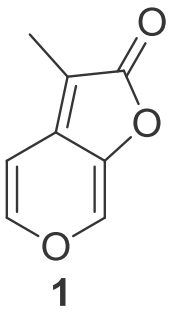

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Fig. 1. Structure of 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one (**1**).

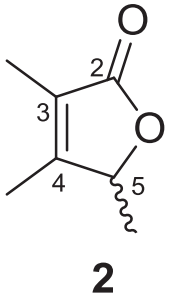

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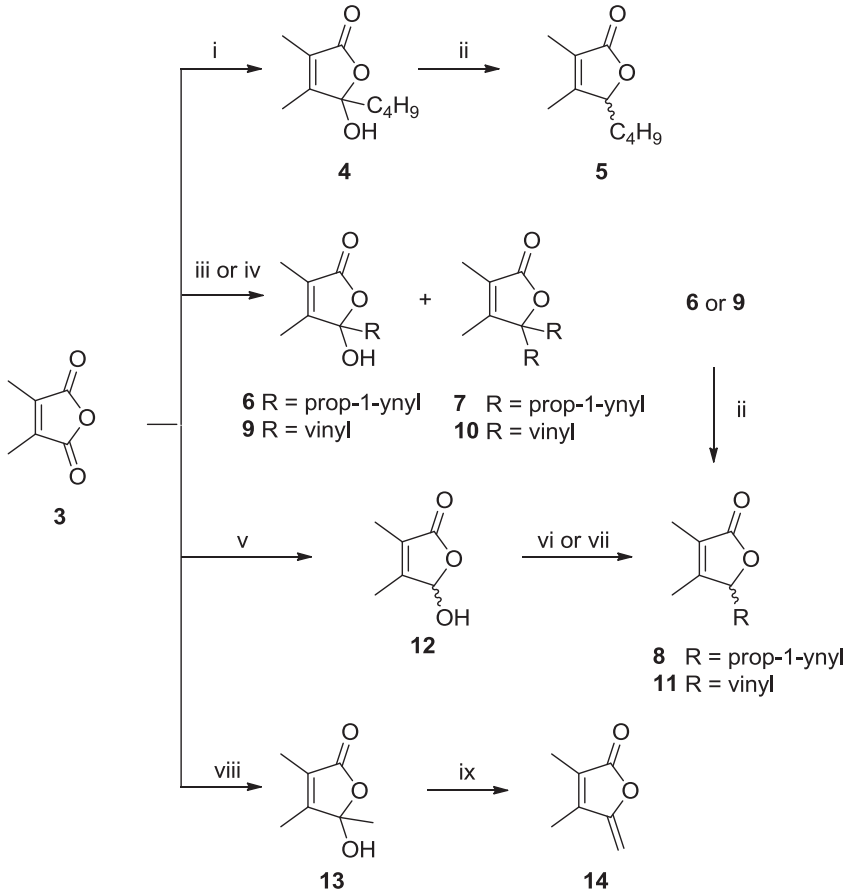
Fig. 2. Structure of 3,4,5-trimethyl-2(5*H*)-furanone (**2**).

Although there has been an intensive effort toward elucidation of the mode of action of KAR_1 (**1**) and structurally-related karrikins (Bythell-Douglas et al., 2013; Flematti et al., 2010; Kagiyama et al., 2013; Nelson et al., 2009, 2010, 2011; Scaffidi et al., 2011, 2012; Soós et al., 2010; Waters et al., 2012; Waters and Smith, 2013), relatively little research has been carried out investigating the action of the germination inhibitor **2**, with the exception of one recent study (Soós et al., 2012), which found that these two compounds are not direct competitors. Thus, in order to further study the action of the naturally occurring inhibitor **2,** we initiated a structure–activity relationship study of synthetic analogs to determine the influence of substituents with differentiated electronic and steric properties to the inhibitory activity. Moreover, substituents were also selected with regard to further functionalization with fluorescent markers. Therefore, several derivatives of **2** were synthesized to investigate the effect of related compounds on the germination inhibition of 'Grand Rapids' lettuce seeds (Scheme 1).

Materials and methods

General methodology and materials

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were measured using a Bruker Avance I – 400 (400 and 100 MHz), and in some cases a Bruker Avance I – 500 (500 and 125 MHz), in $CDCl_3$ with tetramethylsilane as an internal standard. Data are reported as follows: chemical shift in parts per million (δ), multiplicity (*s* = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet or m = multiplet), integration, coupling constant (hertz),



Scheme 1. Reaction conditions: (i) *n*-BuLi, -78 °C; (ii) $NaBH_4$ (5 equiv.), 0 °C, THF/water (24:1); (iii) prop-1-ynylmagnesium bromide, −30 °C; (iv) vinylmagnesium bromide, −78 °C; (v) $LiAlH(t\text{-}BuO)_3$ (1.35 equiv.), −15 °C to r.t., 2 h; (vi) prop-1-ynylmagnesium bromide (2.4 equiv.), 0 °C to r.t. 2 h; (vii) vinylmagnesium bromide (2.4 equiv.), 0 °C to r.t. 2 h; (viii) MeLi, −78 °C; (ix) H_2SO_4/SiO_2, toluene, reflux.