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# More butenolides from plant-derived smoke with germination inhibitory activity against karrikinolide

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ABSTRACT

Plant-derived smoke is known to play an important role in promoting germination in the post-fire environment, thereby stimulating regeneration of vegetation from the soil seed bank following a fire. Several compounds with germination promotory activity have been identified in recent years, with one such compound, 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one (karrikinolide; $KAR_1$) known to be highly active in promoting germination of many plant species at low concentrations. In contrast, a related compound, (5*RS*)-3,4,5-trimethylfuran-2(5*H*)-one, also found in plant-derived smoke, was shown to have an antagonistic effect against $KAR_1$, resulting in lowered germination levels when tested on light-sensitive 'Grand Rapids' lettuce seeds which usually have high germination levels when treated with $KAR_1$. Considering the combination of such types of compounds in smoke, there is the possibility of smoke having a dual regulatory mechanism to control post-fire seedling emergence. Here we report the identification of two structurally related butenolides from plant-derived smoke with germination inhibitory activity, namely 5,5-dimethylfuran-2(5*H*)-one and (5*RS*)-5-ethylfuran-2(5*H*)-one. In the germination test system using light-sensitive 'Grand Rapids' lettuce seeds, the 5,5-dimethylfuran-2(5*H*)-one showed similar levels of activity against $KAR_1$ as previously observed with the (5*RS*)-3,4,5-trimethylfuran-2(5*H*)-one, whereas the (5*RS*)-5-ethylfuran-2(5*H*)-one was approximately 100 times less active.



## 1. Introduction

The interest in understanding the role of smoke in germination, and the intriguing possibility of chemical cues with the ability to promote germination in post fire environments, as was clearly demonstrated by De Lange and Boucher (1990), led to several attempts at isolating and characterising plant-derived smoke compounds (Light et al., 2009; Nelson et al., 2012). Whilst many studies in the 1990s showed that plant-derived smoke is an important environmental cue and promoter of germination for seeds of many species from both fire-prone and non-fire-prone environments (Van Staden et al., 2000; Jefferson et al., 2014), it was not until 2004, that 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one (**1**) (karrikinolide, $KAR_1$) (see Fig. 3 below), a previously unknown compound from plant-derived smoke, was isolated and identified to be a key germination promoter (Flematti et al., 2004; Van Staden et al., 2004). $KAR_1$ was shown to be highly active in promoting seed germination at extremely low concentrations; for some species as low as 1 nM (Flematti et al., 2004; Van Staden et al., 2004; Light et al., 2009). Several subsequent studies have demonstrated the presence of other compounds in smoke that exhibit germination promotory activity, including five related karrikins ($KAR_2$-$KAR_6$) (Flematti et al., 2009), as well as cyanohydrins (Flematti et al., 2011). Consequently, karrikins are now considered to be an important family of plant growth regulators produced during pyrolysis reactions (Chiwocha et al., 2009; Nelson et al., 2012).

In general, smoke provides a positive stimulus for germination of many species, although a negative effect of high concentrations of smoke-water on germination has also been noted (Drewes et al., 1995; Lloyd et al., 2000; Adkins and Peters, 2001; Daws et al., 2007). In our previous experimentation on the isolation of the germination promoter from plant-derived smoke (i.e. $KAR_1$, **1**), light-sensitive 'Grand Rapids' lettuce seeds were used to evaluate germination activity of the test samples for bioactivity-guided fractionation (Drewes et al., 1995; Van Staden et al., 2004). The process involved using liquid-liquid partitioning, vacuum liquid chromatography and column chromatography with Sephadex LH-20 (Van Staden et al., 2004). Following column chromatography using Sephadex LH-20, several fractions showed germination activity of 0%, which was even lower than the control (Fractions 33–45, Fig. 1). This was in contrast to the high germination activity observed in Fractions 56–63 (Fig. 1) from which the germination promoting compound $KAR_1$ (**1**) was isolated (Van Staden et al., 2004). In comparison with the level of seed germination obtained in water controls, which was usually around 20–30%, the surprisingly

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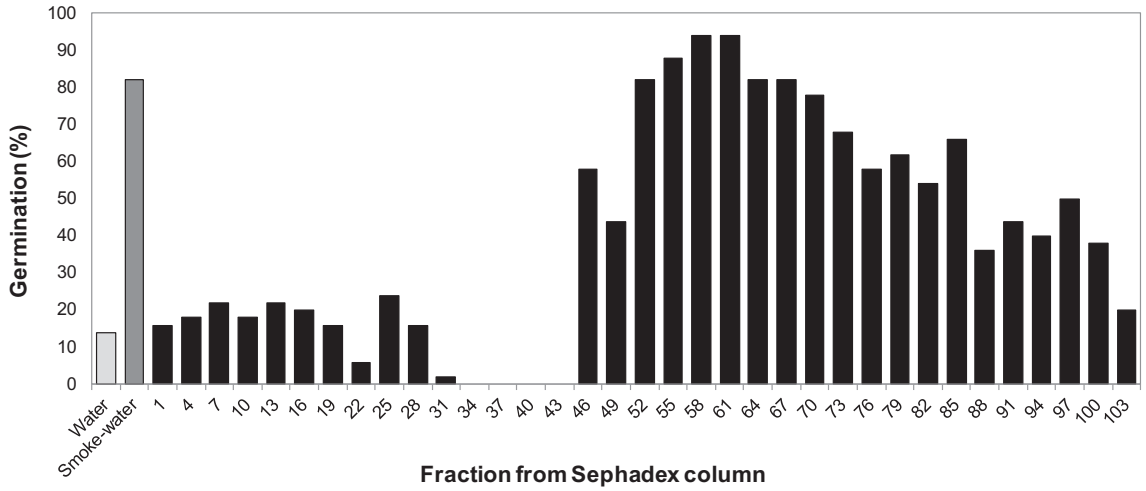


**Fig. 1.** Germination activity testing of fractions collected from Sephadex LH-20 column fractionation of crude smoke-water for the isolation of smoke-derived compounds. The number for each fraction is an arbitrary number allocated to sequential fractions collected at elution rate of 15 mL $h^{-1}$. Germination activity was tested on 'Grand Rapids' lettuce seeds germinated in the dark at 25 °C for 24 h. Light grey bar is for water control; Dark-grey bar is for crude smoke-water control at 1:1000 dilution.

low level of germination associated with Sephadex Fractions 33–45, indicated the possible presence of compounds with germination inhibitory activity in the smoke-water. Further semi-preparative HPLC fractionation of Sephadex Fractions 33–45 yielded HPLC Fraction 15 (eluting between 14 and 16 min) (Fig. 2) from which the germination inhibitor (5*RS*)-3,4,5-trimethylfuran-2(5*H*)-one (**2**; Fig. 3) was identified (Light et al., 2010).

The two enantiomers of this compound were equally effective in significantly reducing the promotive effect of 1 nM $KAR_1$ (**1**) at concentrations of 10 μM (Light et al., 2010). In another study, (5*RS*)-3,4,5-trimethylfuran-2(5*H*)-one (**2**) was similarly shown to reduce the promotory effect of 10 nM $KAR_3$ on 'Grand Rapids' lettuce seed germination at 10–1000 μM (Nair et al., 2014). Whilst the mode of action of **2** is not fully understood, an investigation on the molecular aspects of the effects of **1** and **2** on 'Grand Rapids' lettuce seed germination found that **1** and **2** are not direct competitors (Soós et al., 2012).

In further efforts to understand the action of **2**, synthetic analogues were developed and their activity evaluated in comparison with **2** in terms of their capacity to reduce the germination promoting effect of 0.01 μM $KAR_1$ on the germination of light-sensitive 'Grand Rapids' lettuce seeds in the dark (Pošta et al., 2013; Pošta et al., 2017). In the first structure-activity relationship study, 11 synthetic compounds were tested to determine the influence of substituents with different electronic and steric properties on the inhibitory activity of the compounds. Only (5*RS*)-3,4-dimethyl-5-vinylfuran-2(5*H*)-one (**3**) and 3,4-dimethyl-5,5-divinylfuran-2(5*H*)-one (**4**) showed activity at a similar level as observed with (5*RS*)-3,4,5-trimethylfuran-2(5*H*)-one (**2**) (Pošta et al., 2013). In the second study, with *N*- and *S*-analogues it was found that the sulphur analogue of **2**, (5*RS*)-3,4,5-trimethyl-2(5*H*)-thiophenone (**5**), had germination inhibitory activity comparable to that of **2** (Pošta et al., 2017).

The present study follows on from our previous report in which the butenolide (5*RS*)-3,4,5-trimethylfuran-2(5*H*)-one (**2**) was isolated (Light et al., 2010). The semi-preparative HPLC separation depicted in Fig. 2 indicated that Fraction 11 could possibly contain another germination inhibitor. This fraction was further analysed and here we report on the isolation and identification of two additional butenolides from smoke-water with germination inhibitory activity.

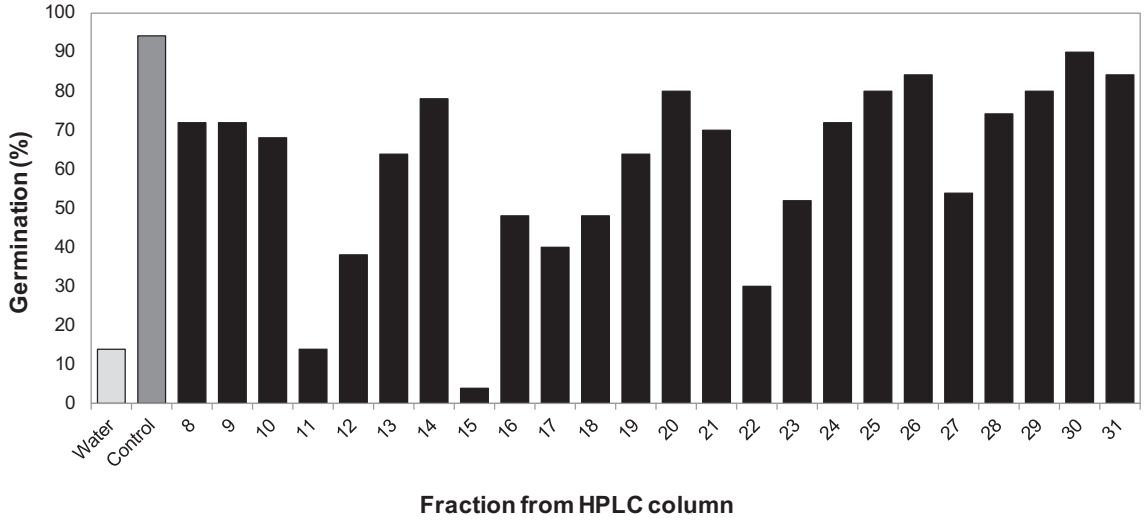


**Fig. 2.** Germination activity testing of fractions collected from HPLC fractionation for the isolation of smoke-derived compounds. The number for each fraction is relative to retention time in min. Germination activity was tested on 'Grand Rapids' lettuce seeds germinated in the dark at 25 °C for 24 h, in combination with $KAR_1$ (**1**, 0.01 μM). Light grey bar is for water control; Dark-grey bar is for $KAR_1$ (**1**, 0.01 μM) control.

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