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Short communication

# Synthesis, X-ray structure determination and germination studies on some smoke-derived karrikins

J.J. Nair [a], M. Pošta [b], H.B. Papenfus [a], O.Q. Munro [c], P. Beier [b], J. Van Staden [a,*]

[a] Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa
[b] Institute of Organic Chemistry and Biochemistry AS CR, v.v.i., Flemingovo nám. 2, 166 10 Prague 6, Czech Republic
[c] School of Chemistry and Physics, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

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ABSTRACT

The discovery of the karrikin class of plant growth regulators (PGRs) is a milestone accomplishment in plant biochemistry and physiology, with significant potential in agriculture and horticulture. These compounds have in common a fused furano-pyran ring system featuring various permutations of methyl substitution. Chief amongst these compounds is karrikinolide ($KAR_1$), identified as the key germination stimulant present in plant-derived smoke, which together with five other closely-related structures ($KAR_2$–$KAR_6$) make up the karrikin class of PGRs. By contrast, the germination inhibitor 3,4,5-trimethyl-2(*5H*)-furanone has also been identified in plant-derived smoke. Various synthetic endeavours have been undertaken for structure–activity relationship study purposes as well as to probe the molecular mechanics of these compounds. In this study, syntheses of $KAR_1$, $KAR_3$ and S-$KAR_1$ were carried out and their structures verified by X-ray crystallography. Effects on germination were measured against the inhibitor 3,4,5-trimethyl-2(*5H*)-furanone in Grand Rapids lettuce seeds. X-ray crystallographic data and germination promotory activity for S-$KAR_1$ are described for the first time.



## 1. Introduction

The elucidation of the role of plant hormones in plant biochemical and physiological processes has had a massive impact on agriculture and horticulture over the past several decades (Osborne and McManus, 2005; Srivastava, 2002). Of these substances, abscisic acid, auxins, cytokinins, ethylene and gibberellins are widely recognized as the five major classes of plant hormones with well-defined functions, often in tandem with each other, in plant growth regulation (Osborne and McManus, 2005; Srivastava, 2002). Apart from these, a further eight chemical entities, including brassinosteroids, salicylic acid, jasmonates, plant peptide hormones, polyamines, nitric oxide, strigolactones and karrikins are also known to exhibit a significant array of plant growth regulatory effects (Brewer et al., 2013; Nelson et al., 2012; Osborne and McManus, 2005; Srivastava, 2002). The discovery of the karrikin class of plant growth regulators (PGRs) has been recent, with the identification of karrikinolide ($KAR_1$) (**1**) (Scheme 1) as the key germination stimulant present in plant-derived smoke only occurring in 2004 (Flematti et al., 2004; Van Staden et al., 2004). This finding was a noteworthy progression from events prior which showed that burnt plant material, as well as aqueous extracts of the burnt material, was able to stimulate germination of several smoke-responsive species (De Lange and Boucher, 1990; Keeley et al., 1985; Wicklow, 1977). As a consequence, several thousand plant species both wild and cultivated, irrespective of fire sensitivity, have been examined for responses to smoke thereby exploiting the benefits of smoke application technology (Brown and Van Staden, 1997; Chiwocha et al., 2009; Light and Van Staden, 2004; Nelson et al., 2012; Van Staden et al., 2000). In addition, the promotory effects of smoke-derived media have also been recorded for other key processes, including flowering, rooting and somatic embryogenesis (Keeley, 1993; Senaratna et al., 1999; Taylor and Van Staden, 1996). Thus, the identification of $KAR_1$ as the predominant germination cue in smoke affords significant new opportunities in agriculture, horticulture, ecology, land management and conservation (Brown and Van Staden, 1997; Chiwocha et al., 2009; Light and Van Staden, 2004; Nelson et al., 2012; Van Staden et al., 2000). For example, Daws et al. (2007) demonstrated the stimulation of germination in several weed seeds by $KAR_1$. From such a finding, an attractive scenario can be envisaged on a commercial scale in which $KAR_1$ could be used to stimulate the dormant seed bank followed by chemical control with herbicide treatment. Initially, $KAR_1$ was shown to stimulate the germination of three smoke responsive species (*Lactuca sativa*, *Conostylis aculeata* and *Stylidium affine*) up to a level similar to that achieved with plant-derived smoke-water (SW) (Flematti et al., 2004). However, in activity it superseded the SW mixture, which typically contains $KAR_1$ at concentrations around 40 μg/L, so that even at 100 ppt it produced 100% germination of Grand Rapids

*Abbreviations:* EI, electron impact; HRMS, high resolution mass spectroscopy; IR, infrared; KAR, karrikin; NMR, nuclear magnetic resonance; PGR, plant growth regulator; ppt, parts per trillion; SW, smoke-water; TMB, trimethylbutenolide.

* Corresponding author. Tel.: +27 332605130.
*E-mail address:* rcpgd@ukzn.ac.za (J. Van Staden).

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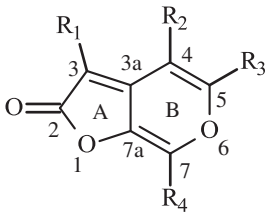
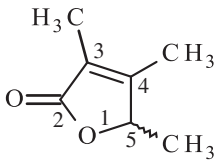
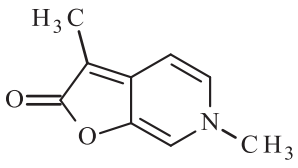


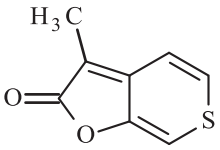
**1.** $R_1$=$CH_3$, $R_2$=$R_3$=$R_4$=H ($KAR_1$)
**2.** $R_1$=$R_2$=$R_3$=$R_4$=H ($KAR_2$)
**3.** $R_1$=$R_3$=$CH_3$, $R_2$=$R_4$=H ($KAR_3$)
**4.** $R_1$=$R_4$=$CH_3$, $R_2$=$R_3$=H ($KAR_4$)
**5.** $R_1$=$R_2$=$R_4$=H, $R_3$=$CH_3$ ($KAR_5$)
**6.** $R_1$=$R_2$=$R_3$=H, $R_4$=$CH_3$ ($KAR_6$)
**8.** $R_1$=$R_3$=$R_4$=$CH_3$, $R_2$=H
(3,5,7-Trimethyl-2*H*-furo[2,3-*c*]pyran-2-one)
**9.** $R_1$=$CH_3$, $R_2$=$R_4$=H, $R_3$=$CH_2OCH_3$
(5-Methoxymethyl-3-methyl-2*H*-furo[2,3-*c*]pyran-2-one)
**10.** $R_1$=$R_4$=$CH_3$, $R_2$=Br, $R_3$=H
(4-Bromo-3,7-dimethyl-2*H*-furo[2,3-*c*]pyran-2-one)



**7.** 3,4,5-Trimethyl-2(5*H*)-furanone (TMB)



**11.** 3-Methylfuro[2,3-*c*]pyridin-2(3*H*)one



**12.** S-$KAR_1$

**Scheme 1.** Compounds related to $KAR_1$ which exhibit diverse germinatory effects.

lettuce seeds (Flematti et al., 2004). Subsequently, a further five closely related compounds ($KAR_2$–$KAR_6$) (**2**–**6**) (Scheme 1), all of which possess a butenolide moiety fused to a pyran ring with differential methyl substitutions, were identified in SW extracts and shown to exhibit significant germination promotory activities (Chiwocha et al., 2009; Flematti et al., 2009; Nelson et al., 2012). Together with $KAR_1$, these compounds now represent the karrikin class of PGRs (Chiwocha et al., 2009; Nelson et al., 2012). In contrast, the *seco*-karrikin derivative 3,4,5-trimethyl-2(5*H*)-furanone (**7**) (also referred to as trimethylbutenolide, TMB), lacking the ring-B pyran moiety, has also been isolated from plant-derived smoke and shown to exhibit significant germination inhibitory effects (Light et al., 2010; Pošta et al., 2013). The potency of TMB (**7**) (Scheme 1) can be gauged from its ability, at various concentrations, to suppress germination of Grand Rapids lettuce seeds in the presence of $KAR_1$ (Light et al., 2010; Pošta et al., 2013). Given the economic potential of karrikins in various agriculture-related sectors as well as their low natural abundance, several novel strategies have been devised towards the synthesis of this group of compounds (Flematti et al., 2005; Goddard-Borger et al., 2007; Matsuo and Shindo, 2011; Nagase et al., 2008; Sun et al., 2008). These efforts have also supplied diverse analogues (such as **8**–**11**) for structure–activity relationship (SAR) studies (Flematti et al., 2005, 2007; Goddard-Borger et al., 2007; Matsuo and Shindo, 2011; Nagase et al., 2008; Sun et al., 2008), which have provided useful insights to the molecular target and mechanism of action of the karrikin class of PGRs (Blythell-Douglas et al., 2013; Chiwocha et al., 2009; Kagiyama et al., 2013; Nelson et al., 2012; Waters and Smith, 2013; Waters et al., 2013; Zhao et al., 2013). In continuation of these efforts, we undertook the synthesis of $KAR_1$ (**1**), $KAR_3$ (**3**) as well as S-$KAR_1$ (**12**) (Scheme 1) and confirmed their structures via single crystal X-ray analysis. In addition, all three compounds were screened for germination activity using Grand Rapids lettuce seeds, with and without the inhibitor TMB (**7**). This response is reported for the first time for the sulphur analogue (S-$KAR_1$) which, together with those observed for $KAR_1$ and $KAR_3$, contributes further to the understanding of the germination promoting effects of karrikins.

## 2. Materials and methods

### 2.1. General

Melting points (uncorrected) were measured on a Gallenkamp melting point apparatus. IR spectra were measured on a Bio-Rad FTS-40 series spectrometer in dry film. EIMS were run on a Micromass Quattro Ultima spectrometer fitted with a direct injection probe (DIP) with ionization energy set at 70 eV and HRMS (EI) were performed with a Micromass Q-Tof Ultima spectrometer. $^1$H and $^{13}$CNMR spectra were recorded on a Bruker AV400 spectrometer in $CDCl_3$, chemical shifts are reported in units of $\delta$ (ppm) and coupling constants (*J*) are expressed in Hz. X-ray crystal structures were acquired with a Bruker *SMART APEX2 DUO* area detector diffractometer. Silica gel Merck KGaA (70–230 mesh) was used for CC and TLC silica gel 60 $F_{254}$ for analytical and preparative TLC (both Merck KGaA). Spots on chromatograms were detected under UV light (254 and 365 nm) and by anisaldehyde reagent stain.

### 2.2. Synthesis of $KAR_1$, $KAR_3$ and S-$KAR_1$

The method of Flematti et al. (2005) was utilized in the preparation of $KAR_1$ (**1**) as well as its sulphur analogue S-$KAR_1$ (**12**) in three steps employing the common starting material pyromeconic acid (**13**) (Scheme 2). Similarly, $KAR_3$ was accessible in similar yields over the same number of steps from 2-methyl pyromeconic acid (details not shown).

### 2.3. X-ray structure analysis

A representative procedure for the X-ray analysis of $KAR_3$ is as described below (Nair et al., 2013), with slight modifications being implemented for $KAR_1$ and S-$KAR_1$ analyses (methods not described). A colourless shard cut from a large crystal of $KAR_3$ with the dimensions 0.25 × 0.30 × 0.30 mm$^3$ was mounted in Paratone® oil using a 200 μm diameter plastic cryo-loop. A total of 1080 frames were collected at 100(2) K with an exposure time of 20 min. Integration of the data using a monoclinic unit cell yielded a total of 12,225 reflections to a maximum θ angle of 25.16° (0.84 Å resolution), of which 2709 were independent (average redundancy 4.513, completeness = 98.3%, $R_{int}$ = 3.25%, $R_{sig}$ = 2.65%) and 2380 (87.86%) were greater than 2σ($F^2$). The final cell constants of $a$ = 7.5701(12) Å, $b$ = 15.115(2) Å, $c$ = 13.440(2) Å, β = 90.046(10)°, volume = 1537.8(4) Å$^3$, are based upon the refinement of the XYZ-centroids of 4442 reflections above 20 σ(I) with 5.381° < 2θ < 49.99°. The ratio of minimum to maximum apparent transmission was 0.915. The structure was solved and refined with OLEX2 software using the space group *P*21/*c*, with Z = 8 for the formula unit $C_9H_8O_3$. CCDC deposits 96,1473, 943,143 and