DOI:10.1021/jf101690a

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Structure−Activity Relationship of Karrikin Germination Stimulants

Gavin R. Flematti,*,† Adrian Scaffidi,† Ethan D. Goddard-Borger,† Charles H. Heath,† David C. Nelson,† Lucy E. Commander,‡,§ Robert V. Stick,† Kingsley W. Dixon,‡,§ Steven M. Smith,† and Emilio L. Ghisalberti†

†School of Biomedical, Biomolecular and Chemical Sciences, The University of Western Australia, Crawley, WA 6009, Australia, ‡Kings Park and Botanic Garden, West Perth, WA 6005, Australia, and §School of Plant Biology, The University of Western Australia, Crawley, WA 6009, Australia

Karrikins (2*H*-furo[2,3-*c*]pyran-2-ones) are potent smoke-derived germination promoters for a diverse range of plant species but, to date, their mode of action remains unknown. This paper reports the structure−activity relationship of numerous karrikin analogues to increase understanding of the key structural features of the molecule that are required for biological activity. The results demonstrate that modification at the C5 position is preferred over modification at the C3, C4, or C7 positions for retaining the highest bioactivity.

KEYWORDS: Karrikinolide; karrikin; seed germination; seed dormancy; smoke; germination stimulant

INTRODUCTION

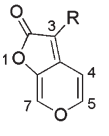
Smoke obtained from the burning of plant material promotes the germination of seeds from at least 1200 plant species hailing from over 80 genera (*1*). In 2004, the structure of the major component of smoke responsible for promoting germination was elucidated (*2*, *3*). This compound was identified as 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one, **1** (**Figure 1**).

Recently, five analogues of **1** have been identified in smoke and demonstrated to have similar, albeit less potent, germination-promoting activity (*4*). This collection of compounds now carry the common name "karrikin", derived from karrik, the traditional word for smoke in the language of the Aboriginal Noongar people, endemic to southwestern Western Australia, where this discovery was made. The most active karrikin, compound **1**, is considered to be the parent compound and carries the name karrikinolide or KAR_1. The karrikins represent a new class of naturally occurring seed germination stimulants that are active in a number of species at concentrations as low as 1 nM (*2*).

Since the discovery of karrikinolide, there has been significant interest in its application within the agricultural and horticultural industries (*1*,*5*,*6*). Karrikinolide has proven to be highly effective at promoting seed germination of species useful for land restoration programs (*7*−*10*) and has been shown to promote the germination of various agricultural weeds (*11*, *12*). Furthermore, karrikinolide can increase seedling vigor in commercially relevant species such as maize (*13*) and rice (*14*) and may even improve yields in tomatoes (*15*).

At present, the mechanism by which karrikinolide promotes seed germination is unknown, although a number of physiological studies have been conducted to investigate smoke- and/or karrikinolide-stimulated germination. Early work demonstrated that smoke could influence the way that seeds respond to light and gibberellic acid (GA). For example, smoke substitutes for light in the germination of light-sensitive lettuce seeds (*Lactuca sativa* L. cv. Grand Rapids) (*16*), and germination is comparable to the results obtained when seeds are treated with GA_3. Furthermore, smoke-induced germination in cv. Grand Rapids is reduced by exposure to gibberellin biosynthesis inhibitors such as paclobutrazol (*17*). Likewise, it has been shown that karrikinolide can act in a similar fashion to GA_3 in promoting the germination of light-sensitive Asteracea species (*7*) and that both light and gibberellin biosynthesis are required for karrikinolide to elicit germination in seeds of primary dormant *Arabidopsis thaliana* (*18*, *19*). Previous studies have suggested that smoke, and now karrikinolide, may promote germination by stimulating GA biosynthesis and reduce levels of abscisic acid (ABA) (*20*), an inhibitor of germination (*21*). However, analysis of GA_4 and ABA levels in *A. thaliana* following treatment with karrikinolide did not support this hypothesis. GA_4 and ABA levels did not change relative to control levels during the pregermination period (*18*).

To further explore the mode of action of karrikinolide, a number of techniques could be used. The use of various probes to label molecules of biological interest has become a popular



1: R = CH_3
2: R = H
3: R = CH_2CH_3
4: R = $CH_2CH_2CH_3$
5: R = CH_2OH
6: R = CH_2OCH_3
7: R = CHO
8: R = $COCH_3$
9: R = CHNOH
10: R = CHF_2
11: R = C_6H_5
12: R = CN
13: R = NO_2
14: R = Cl
15: R = Br
16: R = I

Figure 1. Structure of karrikinolide (**1**) and various C3-substituted karrikins.

*Corresponding author (phone +61 8 6488 4461; fax +61 8 6488 7330; e-mail gavin.flematti@uwa.edu.au).

 Published on Web 07/09/2010

approach in chemical biology. However, labeling of **1** would be useful only if the resulting compound retained activity as a germination promoter. Thus, some knowledge of structure−activity relationships for the karrikins would aid in further mode of action studies. Up to this point, only a small number of karrikinolide derivatives have been prepared and tested as germination stimulants (*22*). Here, the germination activity of a range of new and previously prepared karrikin analogues is described, and some emerging structure−activity relationships are outlined.

MATERIALS AND METHODS

General. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on a Bruker ARX500 (500 MHz for δ_H and 125.7 MHz for δ_C) or on a Bruker AV600 (600 MHz for δ_H and 150.9 MHz for δ_C) spectrometer. Unless otherwise stated, deuterochloroform ($CDCl_3$) was used as the solvent with residual $CHCl_3$ (δ_H 7.26) or $CDCl_3$ (δ_C 77.0) being employed as internal standard. Spectra run in hexadeuteroacetone ($(CD_3)_2CO$) used residual $(CH_3)_2CO$ (δ_H 2.04) or $(CD_3)_2CO$ (δ_C 29.8) as internal standard. Melting points (mp) were determined on a Reichert hot-stage apparatus. High-resolution mass spectra (HR-MS) were recorded with a VG-Autospec spectrometer using the fast atom bombardment (FAB) technique or electron impact (70 eV) ionization (EI).

All experiments were carried out under an inert atmosphere, and all solvents were dried prior to use. A "usual workup" refers to dilution with water, repeated extraction into an organic solvent, and sequential washing of the combined extracts with hydrochloric acid (1 M, where appropriate), saturated sodium bicarbonate, and brine solutions, followed by drying over anhydrous magnesium sulfate, filtration, and evaporation of the solvent by means of a rotary evaporator under reduced pressure. Flash chromatography was performed on Merck silica gel 60 with the specified solvents. Thin-layer chromatography (TLC) was effected on Merck silica gel 60 F254 aluminum-backed plates that were stained by heating (> 200 °C) with 0.25 M ceric sulfate in 2 M sulfuric acid. Percentage yields for chemical reactions as described are quoted for only those compounds that were purified by recrystallization or by chromatography and for which the purity was assessed by TLC or NMR spectroscopy.

Seed Bioassay. All germination experiments were performed using *Solanum orbiculatum* seeds collected in the Shark Bay region (Western Australia) and stored at −80 °C until use. All assays were conducted using Millipore (MP) water obtained by filtration through a Milli-Q ultrapure water system (Millipore, Australia). A 1% acetone solution was used as a control. Stock solutions of 100 ppm were prepared by dissolving 1.0 mg of compound in 100 μL of acetone prior to the addition of 9.9 mL of MP water. Subsequent dilutions with Milli-Q water gave concentrations of 10 ppm, 1 ppm, 100 ppb, 10 ppb, and 1 ppb. The solutions were tested for germination activity by adding 2.5 mL to two layers of Whatman no. 1 filter paper (7.0 cm) in plastic Petri dishes (9.0 cm) followed by approximately 20−30 seeds. The Petri dishes were sealed with a layer of plastic wrap and stored in a light-proof container for 6 days at 20 ± 1 °C. All experiments were conducted in triplicate.

Synthesis. *4-Ethyl-3-methyl-2H-furo[2,3-c]pyran-2-one* (***20***). Titanium tetrachloride (1.7 mL, 15 mmol) was added to a stirred solution of **21** (1.3 g, 7.0 mmol), tributylamine (3.9 mL, 21 mmol), and methyl pyruvate (1.4 g, 14 mmol) in dichloromethane (20 mL) at −60 °C, and the resulting solution was stirred under an atmosphere of argon (1.5 h). The usual workup (ethyl acetate) followed by flash chromatography (10% ethyl acetate−hexane) gave a pale yellow oil (1.0 g). This oil was dissolved in acetonitrile (15 mL) followed by the addition of triethylamine (590 mg, 5.8 mmol), trifluoroacetic anhydride (900 μL, 6.5 mmol), and 4-dimethylaminopyridine (20 mg, 0.2 mmol) at 0 °C. The solution was stirred (3 h) before being subjected to the usual workup (ethyl acetate) to give a yellow residue that was dissolved in acetonitrile (20 mL) and treated with 1,8-diazabicycloundec-7-ene (800 mg, 5.3 mmol) at 0 °C. This mixture was stirred (2 h) followed by the usual workup to return a brown residue. *p*-Toluenesulfonic acid (870 mg, 4.6 mmol) was added to the brown residue in toluene (25 mL) and the resulting mixture refluxed under an atmosphere of argon (2 h). The reaction was cooled followed by the usual workup (ethyl acetate), and flash chromatography (20% ethyl acetate−hexane) afforded the butenolide **20** as pale yellow needles (50 mg, 4%): 111−113 °C; δ_H (600 MHz) 7.45 (s, 1H, H7), 7.11 (s, 1H, H5), 2.60 (q, $J_{8,9}$ = 7.3 Hz, 2H, H8), 2.10 (s, 3H), 1.25 (t, 3H, H9); δ_C (150.9 MHz) 171.7 (C2), 144.1 (C5), 141.5, 139.8 (C3a, C7a), 126.5 (C7), 120.1 (C4), 100.1 (C3), 21.5 (C8), 13.8 (C9), 8.9 (C3b); *m*/*z* (EI) 178.0636, $(M)^+$ requires 178.0630.

Dihydro-5-ethyl-6-isopropoxy-2H-pyran-3-(4H)-one (***21***). Tin(IV) chloride (1.0 M in dichloromethane, 300 μL, 0.3 mmol) and isopropanol (3.0 mL) were added to the acetate **19** (*23*) (1.0 g, 5.4 mmol) in dichloromethane (50 mL), and the resulting solution was left to stand (3 h). The reaction was quenched with a saturated sodium bicarbonate solution (50 mL) followed by the usual workup (ethyl acetate) and flash chromatography (10% ethyl acetate−hexane) to yield **21** as a colorless oil (500 mg, 50%, diastereomixture 5:7): δ_H (600 MHz) 4.92 (d, $J_{5,6}$ = 2.8 Hz, 1H, H6), 4.77 (d, $J_{5',6'}$ = 2.8 Hz, 1H, H6′), 4.18 (d, $J_{2',2'}$ = 17.1 Hz, 1H, H2′), 4.14 (d, $J_{2,2}$ = 16.3 Hz, 1H, H2), 4.02−3.94 (m, 2H, H7, H7′), 3.91 (d, 1H, H2), 3.85 (d, 1H, H2′), 2.54 (dd, $J_{4',4'}$ = 15.8, $J_{4',5'}$ = 4.9 Hz, 1H, H4′), 2.47−2.36 (m, 2H, H4, H4), 2.29 (dd, $J_{4',5'}$ = 9.8 Hz, 1H, H4′), 2.02−2.10 (m, H5), 1.83−1.90 (m, H5′), 1.85−1.25 (m, 4H, H9, H9, H9′, H9′), 1.24 (d, $J_{7,8}$ = 6.2 Hz, 3H, H8), 1.23 (d, $J_{7',8'}$ = 6.2 Hz, 3H, H8′), 1.19 (d, $J_{7',8'}$ = 6.2 Hz, 3H, H8′), 1.18 (d, $J_{7,8}$ = 6.2 Hz, 3H, H8), 0.95 (t, $J_{9',10'}$ = 7.4 Hz, 3H, H10′), 0.90 (t, $J_{9,10}$ = 7.5 Hz, 3H, H10); δ_C (150.9 MHz) 210.6 (C3′), 207.8 (C3), 99.3 (C6′), 95.6 (C6), 69.2 (C7′), 69.2 (C7), 67.2 (C2), 66.8 (C2′), 41.4 (C5), 40.8 (C5′), 39.8 (C4), 39.6 (C4′), 25.7 (C9′), 24.4 (C9), 23.4 (C8), 23.1 (C8′), 21.5 (C8′), 21.4 (C8), 11.2 (C10′), 11.0 (C10); *m*/*z* (FAB) 127.0761, $(M + H)^+$ requires 127.0759.

General Procedure for the Preparation of Ether Derivatives of 5-Hydroxymethyl-3-methyl-2H-furo[2,3-c]pyran-2-one (***26***) *and 7-Hydroxymethyl-3-methyl-2H-furo[2,3-c]pyran-2-one* (***33***). Sodium hydride (60% dispersion in mineral oil, 2 mmol) was added to the alcohol (1 mmol) and a bromide (1−10 mmol) in dimethylformamide (15 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred (0.5−4.5 h). The mixture was cooled to 0 °C and quenched by the dropwise addition of 2 M HCl. The usual workup (dichloromethane) followed by flash chromatography afforded the appropriate ether.

5-Butoxymethyl-3-methyl-2H-furo[2,3-c]pyran-2-one (***27***). The reaction using **26** (*24*) (18 mg, 0.10 mmol) and 1-bromobutane (0.1 mL, 0.9 mmol) was carried out according to the general procedure (4.5 h). Flash chromatography (20% ethyl acetate−hexane) yielded the ether **27** as a light yellow wax (7 mg, 35%): δ_H (500 MHz, $(CD_3)_2CO$) 7.77 (s, 1H, H7) 6.80 (s, 1H, H4), 4.35 (s, 2H, H8), 3.55 (t, J = 6.5 Hz, 2H, H10), 1.88 (s, 3H, H3b), 1.62−1.57 (m, 2H, H11), 1.41 (m, 2H, H12), 0.91 (t, J = 7.4 Hz, 3H, H13); δ_C (125.8 MHz, $(CD_3)_2CO$) 171.3 (C2), 159.3 (C5), 142.6, 141.5 (C3a, C7a), 127.5 (C7), 101.1 (C4), 100.2 (C3), 71.5 (C8), 69.4 (C10), 32.4 (C11), 19.9 (C12), 14.1 (C13), 7.6 (C3b); *m*/*z* (EI) 236.1049, $(M)^+$ requires 236.1049.

5-Heptoxymethyl-3-methyl-2H-furo[2,3-c]pyran-2-one (***28***). The reaction using **26** (*24*) (35 mg, 0.20 mmol) and 1-bromoheptane (0.2 mL, 1.3 mmol) was carried out according to the general procedure (0.5 h). Flash chromatography (20% ethyl acetate−hexane) furnished the ether **28** as a colorless wax (12 mg, 22%): δ_H (500 MHz) 7.41 (s, 1H, H7), 6.55 (s, 1H, H4), 4.28 (s, 2H, H8), 3.55 (t, J = 6.6 Hz, 2H, H10), 1.89 (s, 3H, H3b), 1.61 (tt, $J_{10,11}$, $J_{11,12}$ = 6.6 Hz, 2H, H11), 1.41−1.25 (m, 8H, H12, H13, H14, H15), 0.90 (m, 3H, H16); δ_C (125.8 MHz) 171.6 (C2), 157.9 (C5), 142.0, 141.0 (C3a, C7a), 126.4 (C7), 100.5, 100.5 (C3, C4), 72.0 (C8), 69.0 (C10), 31.9, 29.7, 29.2, 26.2, 22.7, 14.2 (C11, C12, C13, C14, C15, C16), 7.9 (C3b); *m*/*z* (FAB) 279.1592, $(M + H)^+$ requires 279.1596.

5-Dodecoxymethyl-3-methyl-2H-furo[2,3-c]pyran-2-one (***29***). The reaction using **26** (*24*) (27 mg, 0.15 mmol) and 1-bromododecane (0.2 mL, 0.8 mmol) was carried out according to the general procedure (0.5 h). Flash chromatography (10% ethyl acetate−hexane) returned the ether **29** as a colorless wax (9 mg, 17%): δ_H (500 MHz) 7.41 (s, 1H, H7), 6.55 (s, 1H, H4), 4.28 (s, 2H, H8), 3.55 (t, $J_{10,11}$ = 6.7 Hz, 2H, H10), 1.89 (s, 3H, H3b), 1.65 (tt, $J_{11,12}$ = 6.7 Hz, 2H, H11), 1.40−1.22 (m, 18H), 0.90 (t, $J_{20,21}$ = 7.0 Hz, 3H, H21); δ_C (125.8 MHz) 171.6 (C2), 157.9 (C5), 142.0, 140.8 (C3a, C7a), 126.4 (C7), 100.5 (C3, C4), 72.0 (C8), 69.0 (C10), 32.1, 29.8, 29.7, 29.6, 29.5, 14.3 (C11−21), 7.9 (C3b); *m*/*z* (FAB) 349.2344, $(M + H)^+$ requires 349.2379.

5-Benzyloxymethyl-3-methyl-2H-furo[2,3-c]pyran-2-one (***30***). The reaction using **26** (*24*) (40 mg, 0.22 mmol) and benzyl bromide (0.2 mL,

1.7 mmol) was carried out according to the general procedure (0.5 h). Flash chromatography (30% ethyl acetate−hexane) yielded the ether **30** as a light orange oil (26 mg, 42%): δ_H (500 MHz, $(CD_3)_2CO$) 7.76 (s, 1H, H7) 7.25−7.45 (m, 5H, Ar), 6.85 (s, 1H, H4), 4.67 (s, 2H, H8/H10), 4.42 (s, 2H, H10/H8), 1.92 (s, 3H, H3b); δ_C (125.8 MHz, $(CD_3)_2CO$) 171.3 (C2), 158.9 (C5), 142.6, 141.4 (C3a, C7a), 138.8 (C11) 129.2, 128.7, 128.6, 127.6 (C7, Ar), 101.5 (C4), 100.3 (C3), 73.5, 68.9 (C8, C10), 7.7 (C3b); *m*/*z* (EI) 270.0890, $(M)^+$ requires 270.0892.

5-(3-Phenoxypropoxy)methyl-3-methyl-2H-furo[2,3-c]pyran-2-one (***31***). The reaction using **26** (*24*) (44 mg, 0.24 mmol) and 3-phenoxypropyl bromide (0.2 mL, 1.3 mmol) was carried out according to the general procedure (4 h). Flash chromatography (10% diethyl ether−toluene) afforded the ether **31** as a pale yellow wax (12 mg, 16%): δ_H (600 MHz) 7.36 (s, 1H, H7), 7.30−7.26, 6.96−6.87 (2 m, 5H, Ar), 6.53 (d, $J_{4,8}$ = 0.6 Hz, 1H, H4), 4.30 (d, 2H, H8), 4.10 (t, $J_{11,12}$ = 6.0 Hz, 2H, H12), 3.75 (t, $J_{10,11}$ = 6.1 Hz, 2H, H10), 2.11 (tt, 2H, H11), 1.89 (s, 3H, H3b); δ_C (125.8 MHz) 171.6 (C2), 158.9, 157.5 (C5, C14), 141.9, 140.7 (C3a, C7a), 129.6, 121.0, 114.5 (Ar), 126.4 (C7), 100.7, 100.5 (C3, C4) 69.3 (C8), 68.0 (C10), 64.2 (C12), 29.8 (C11), 7.8 (C3b); *m*/*z* (FAB) 315.1250, $(M + H)^+$ requires 315.1232.

7-Hydroxymethyl-3-methyl-2H-furo[2,3-c]pyran-2-one (***33***). Aluminum-(III) chloride (480 mg, 3.6 mmol) was added to the ester **32** (*25*) (240 mg, 1.2 mmol) and *tert*-butylamine borane (640 mg, 7.2 mmol) in dichloromethane (25 mL), and the mixture was stirred at 40 °C (15 min). The reaction was poured into ice-cold 1 M HCl (50 mL) followed by the usual workup (dichloromethane) and flash chromatography (50−70% ethyl acetate−hexane) to afford the alcohol **33** as light tan needles (180 mg, 87%): 160−162 °C; δ_H (600 MHz) 7.35 (d, $J_{4,5}$ = 5.5 Hz, 1H, H5), 6.48 (d, 1H, H4), 4.68 (s, 2H, H8), 1.94 (s, 3H, H3b); δ_C (150.9 MHz) 170.9 (C2), 147.5 (C5), 139.9, 138.5, 136.5 (C3a, C7, C7a), 103.1, 101.2 (C3, C4), 56.8 (C8), 7.7 (C3b); *m*/*z* (EI) 180.0422, $(M)^+$ requires 180.0423.

7-Methoxymethyl-3-methyl-2H-furo[2,3-c]pyran-2-one (***34***). The reaction using **33** (50 mg, 0.26 mmol) and iodomethane (0.1 mL, 1.6 mmol) was carried out according to the general procedure (1 h). Flash chromatography (30% ethyl acetate−hexane) yielded the ether **34** as a tan powder (37 mg, 68%): mp 86−88 °C; δ_H (600 MHz) 7.35 (d, $J_{4,5}$ = 5.5 Hz, 1H, H5) 6.50 (d, 1H, H4), 4.47 (s, 2H, H8), 3.42 (s, 3H, H10), 2.01 (s, 3H, H3b); δ_C (150.9 MHz) 170.9 (C2), 148.0 (C5), 140.0, 139.7 (C3a, C7a), 134.4 (C7), 103.0 (C4), 101.3 (C3), 65.4 (C8), 58.6 (C10), 7.8 (C3b); *m*/*z* (EI) 194.0585, $(M)^+$ requires 194.0579.

7-Butoxymethyl-3-methyl-2H-furo[2,3-c]pyran-2-one (***35***). The reaction using **33** (50 mg, 0.26 mmol) and 1-bromobutane (0.2 mL, 1.8 mmol) was carried out according to the general procedure (0.5 h). Flash chromatography (20% ethyl acetate−hexane) yielded the ether **35** as a colorless gum (17 mg, 26%): δ_H (600 MHz, $(CD_3)_2CO$) 7.64 (d, $J_{4,5}$ = 5.5 Hz, 1H, H5) 6.77 (d, 1H, H4), 4.47 (s, 2H, H8), 3.52 (t, $J_{10,11}$ = 6.5 Hz, 2H, H10), 1.88 (s, 3H, H3b), 1.57−1.52, 1.39−1.34 (2*m*, 4H, H11, H12), 0.90 (t, $J_{12,13}$ = 7.5 Hz, 3H, H13); δ_C (150.9 MHz, $(CD_3)_2CO$) 169.9 (C2), 148.8 (C5), 139.9, 139.5 (C3a, C7a), 135.1 (C7), 102.8 (C4), 100.0 (C3), 70.1 (C8), 63.5 (C10), 31.4, 18.8, 13.1 (C11, C12, C13), 6.8 (C3b); *m*/*z* (EI) 236.1052, $(M)^+$ requires 236.1049.

7-Benzyloxymethyl-3-methyl-2H-furo[2,3-c]pyran-2-one (***36***). The reaction using **33** (30 mg, 0.2 mmol) and benzyl bromide (0.1 mL, 0.8 mmol) was carried out according to the general procedure (0.5 h). Flash chromatography (30% diethyl ether−hexane) yielded the ether **36** as a colorless gum (12 mg, 30%): δ_H (600 MHz) 7.35−7.26 (m, 6H, H7, Ar), 6.74 (d, $J_{4,5}$ = 5.5 Hz, 1H, H4), 4.59, 4.55 (2s, 4H, H8, H10), 1.92 (s, 3H, H3b); δ_C (150.9 MHz) 170.9 (C2), 147.9 (C5), 139.8, 137.0, 134.7 (C3a, C7, C7a), 139.8, 128.4, 127.9, 127.8 (Ar), 103.0, 101.2 (C3, C4), 73.0, 63.2 (C8, C10), 7.7 (C3b); *m*/*z* (EI) 270.0893, $(M)^+$ requires 270.0892.

RESULTS AND DISCUSSION

The compounds described herein were evaluated as germination stimulants using the seeds of *Solanum orbiculatum* (Solanaceae, Dunal ex Poir.). *S. orbiculatum* is a smoke-responsive species native to Western Australia (*8*) and provides an ideal species for testing analogues as it is highly sensitive to the stimulatory effects of **1** and provides a very low control germination yield (*22*).

C3-Substituted Analogues and Their Germination Activity. Two recent synthetic routes have been reported that provide the desmethyl analogue **2** directly in gram quantities (*24*, *25*), a substantial improvement on the original synthesis from pyromeconic acid (*26*). It has been demonstrated that the C3 position is activated toward electrophilic substitution (*24*), which opens the door to a number of 3-substituted analogues. These analogues can be further modified to yield a small suite of compounds with varied steric and electronic effects.

Previous work had demonstrated that removal of the methyl group of **1** at C3 reduces germination activity by around 2 orders of magnitude (*22*). Replacing the methyl group at C3 with larger alkyl substituents similarly reduced the potency of the resulting compounds, as observed for the ethyl **3** (*24*) and propyl **4** (*24*) derivatives (**Figures 1** and **2**). The ethyl analogue **3** appeared to be an order of magnitude more active than the slightly larger propyl derivative **4**, but was still at least 100 times less potent than the methyl-bearing karrikinolide (**1**). Analogues oxygenated at C3, such as the hydroxymethyl **5** (*24*) and methoxymethyl **6** (*24*), also possessed reduced activity relative to karrikinolide (**Figure 2**).

Compounds with electron-withdrawing groups at C3 have greatly diminished germination activity. The aldehyde **7** (*24*), methyl ketone **8** (*24*), oxime **9** (*24*), and difluoromethyl analogue **10** (*24*) are far less active than karrikinolide, promoting germination only at the highest concentration tested (**Figure 2**). Given the similar van der Waals radii of fluorine and hydrogen, the relatively poor activity of the difluoromethyl analogue **10** (*24*) with respect to karrikinolide is particularly telling of this apparent electronic effect.

The phenyl **11** (*25*), cyano **12** (*24*), and nitro **13** (*24*) analogues are inactive at the concentrations tested (**Figure 2**). Compound **11** is probably inactive because the phenyl group is simply too "bulky", whereas the strongly electron-withdrawing groups of the cyano **12** and nitro **13** analogues appear to abolish biological activity. The chloride **14** (*25*) has appreciable germination-promoting activity. However, substitution in line with the periodical halogen atom order (Cl, Br, and I) at C3 results in progressively weaker biological activity, as observed for the bromide **15** (*25*) and iodide **16** (*25*) (**Figure 2**).

Overall, the natural stimulant karrikinolide is at least an order of magnitude more potent than any C3-modified compound tested here. Thus, it would appear that a methyl group at this position is optimal for germination-promoting activity.

C4-Substituted Analogues and Their Germination Activity. The only analogue prepared so far with substitution at C4 is the 4-bromo-3,7-dimethyl derivative **17** (*22*) (**Figure 3**). The germination activity of **17** was found to be comparable to that of the 3,7-dimethyl analogue **18** (*22*) (**Figure 3**); thus, investigation into other C4-substituted analogues seemed to be appropriate. The synthesis of karrikinolide recently developed by Nagase et al. (*27*) provided a convenient route to C4-substituted analogues by modifying the starting pyranone. The use of the known acetate **19** (*23*) provided the 4-ethyl derivative **20**, via the acetal **21** (**Figure 4**).

Surprisingly, compound **20** was found to have very poor germination-promoting activity (**Figure 5**). With such a simple modification resulting in such a dramatic loss in activity, no further C4-substituted analogues were investigated.

C5-Substituted Analogues and Their Germination Activity. Two C5-substituted compounds, the 3,5-dimethyl **22** (*22*) and 5-methoxymethyl-3-methyl **23** (*22*) analogues (**Figure 6**), have previously demonstrated good germination-promoting activity (*22*).

Recently, an efficient synthetic route to the 5-methoxycarbonyl analogue **24** (*24*) was reported (**Figure 7A**). Whereas the activity of **24** (*24*) itself is poor (**Figure 5**), which is not surprising given the

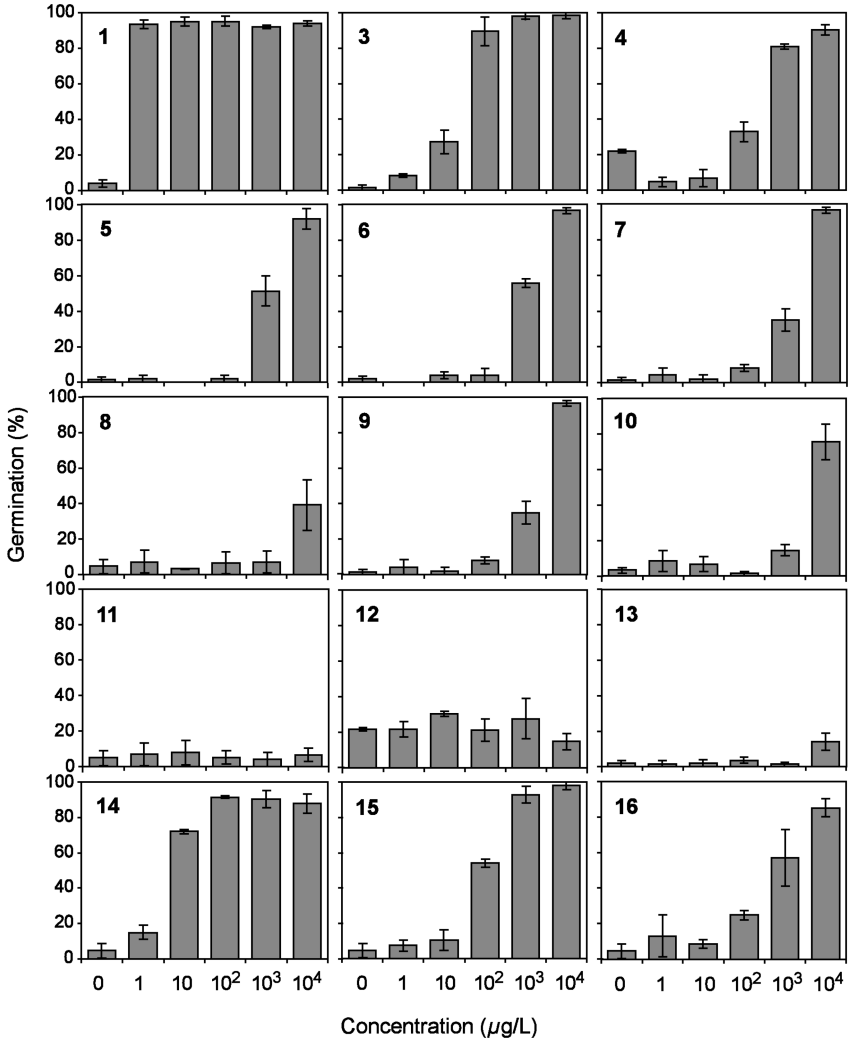


Figure 2. Germination activity of C3-substituted karrikins.

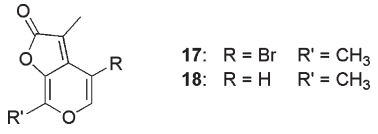


Figure 3. Structure of the 4-bromo-3,7-dimethyl analogue **17** and 3,7-dimethyl analogue **18**.

absence of the methyl group at C3, it has been demonstrated to be an excellent synthon for the preparation of C5-substituted analogues (*24*).

The alcohol **25** (*24*) and the 5-hydroxymethyl-3-methyl analogue **26** (*24*) (**Figure 6**), both prepared from **24**, exhibited limited germination-promoting activity (**Figure 5**). Given that the previously tested methoxymethyl analogue **23** exhibited high germination activity (*22*), additional analogues were prepared by converting **26** into a series of simple ether derivatives (**Figure 7A**). The *n*-butoxymethyl analogue **27** was found to be highly active and comparable to karrikinolide (**Figure 5**). Extension of the alkyl chain length reduced activity significantly, as observed for the *n*-heptoxymethyl **28** and *n*-dodecoxymethyl **29** derivatives (**Figure 5**). The benzyloxymethyl derivative **30** exhibited good activity (**Figure 5**) and demonstrated that some steric bulk is tolerated at the C5 position, although this result is clouded by the fact that the phenoxypropyl ether **31** showed no activity at the concentrations tested (**Figure 5**).

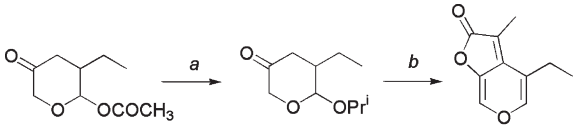


Figure 4. Synthesis of C4-substituted karrikins: (a) $SnCl_4$, Pr^iOH, CH_2Cl_2; (b) (i) $TiCl_4$, Bu_3N, methyl pyruvate, CH_2Cl_2, (ii) $(CF_3CO)_2O$, Et_3N, DMAP, CH_3CN, (iii) DBU, CH_3CN, (iv) *p*-TsOH, $PhCH_3$.

The low activity of the hydroxymethyl derivative **26** (**Figure 5**), when contrasted against the 5-methyl and 5-alkoxymethyl derivatives, suggests that germination activity is reduced when a hydrophilic substituent is present at the C5 position. Also noteworthy is the fact that the *n*-butoxymethyl derivative **27** is significantly more active than the previously prepared 5-methoxy methyl derivative **23** (*22*), indicating lipophilic substituents are well tolerated at this position. Despite the fact that a reduction in germination activity is observed when large alkyl groups are installed at C5, this position can clearly tolerate modification better than C3 and C4.

C7-Substituted Analogues and Their Germination Activity. Earlier work demonstrated that analogues substituted with a methyl group at C7 had significantly reduced germination activity compared to **1** (*22*). Further investigations were conducted.

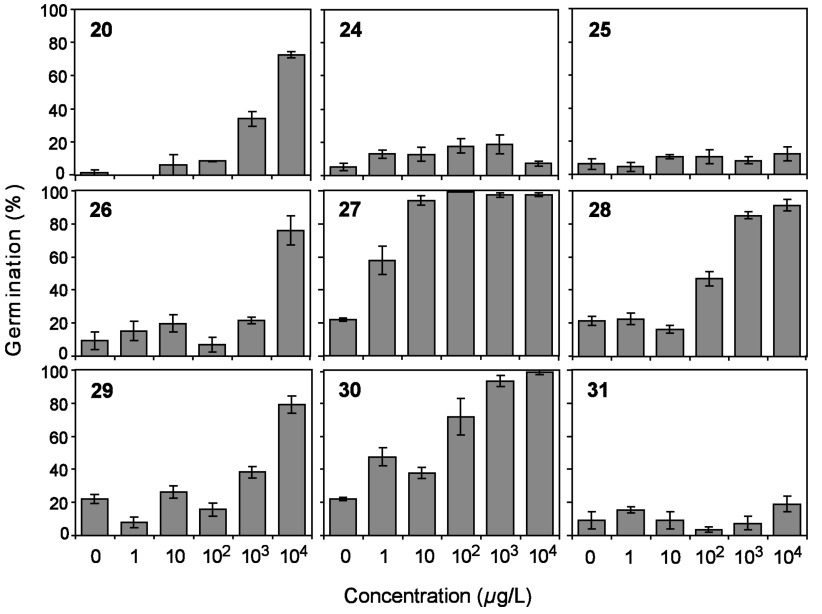
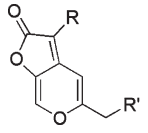


Figure 5. Germination activity of C4- and C5-substituted karrikins.



22: R = CH_3 R' = H
23: R = CH_3 R' = OCH_3
25: R = H R' = OH
26: R = CH_3 R' = OH

Figure 6. Structure of C5-substituted karrikins.

The 7-methylester **32** (*25*) showed good activity, whereas the 7-hydroxymethyl analogue **33**, derived from **32** (**Figure 7B**), showed only activity at the highest concentration tested (**Figure 8**). This result is analogous to that seen at C5, where the hydroxymethyl group was also poorly tolerated.

Several ethers were prepared from the alcohol **33**, with the expectation that some activity may be reclaimed, as observed in the case of the C5-substituted compounds. Thus, the alcohol **33** was converted into the 7-methoxymethyl **34**, *n*-butoxymethyl **35**, and benzyloxymethyl **36** analogues in good yield (**Figure 7B**). The *n*-butoxymethyl **35** and the benzyloxymethyl **36** proved to be an order of magnitude less potent than the corresponding C5 analogues, compounds **27** and **30**, respectively (**Figure 8**).

Surprisingly, the 7-trimethylsilyl derivative **37** (*25*) (**Figure 7B**) showed high germination-promoting activity that was comparable to that of karrikinolide (**1**) (**Figure 8**). However, it seems likely that compound **37** may undergo spontaneous hydrolysis to liberate karrikinolide under the conditions of the assay (*28*), which may explain its potent germination activity.

Knowledge of karrikin structure−activity relationships is valuable for the design of karrikin-based molecular probes for mode of action studies. To this end, the structure of karrikinolide provides only four carbons that can be modified. The results presented here demonstrate that karrikins modified at the C3 position have reduced activity compared with the methyl-bearing karrikinolide, indicating that the methyl substituent at C3 is important for biological activity. Compounds substituted at the C4 position showed significantly impaired bioactivity. However, modifications at C5 and C7 were better tolerated, with compounds modified at C5 found to retain the highest bioactivity. In terms of labeling, compound **26** provides an excellent scaffold for preparing analogues to investigate the karrikinolide mode of action with C5 derivatives reported here demonstrating a high level of bioactivity with *S. orbiculatum*.

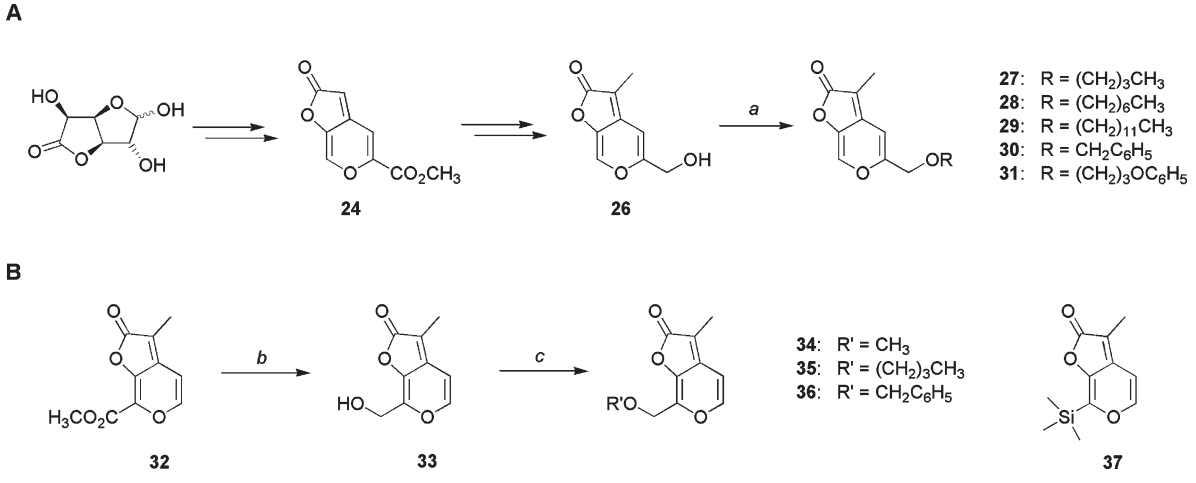


Figure 7. Synthesis of (**A**) C5-substituted karrikins and (**B**) C7-substituted karrikins: (a) NaH, RX, DMF; (b) $tBuNH_2 \cdot BH_3$, $AlCl_3$, CH_2Cl_2; (c) NaH, R′X, DMF.

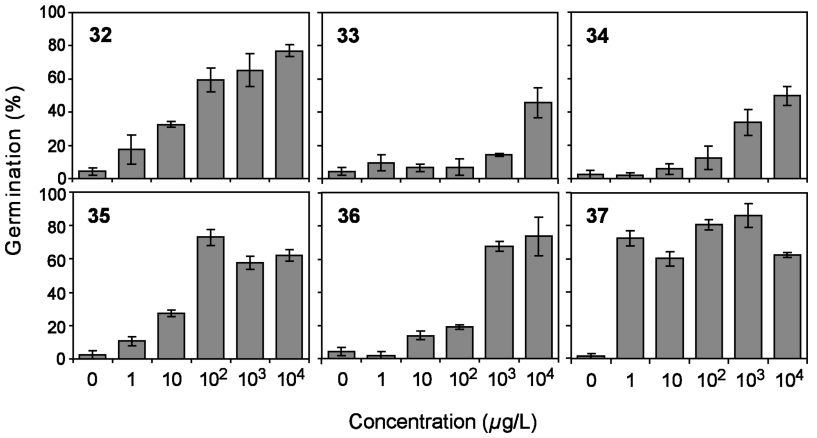


Figure 8. Germination activity of C7-substituted karrikins.

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Received for review May 4, 2010. Revised manuscript received June 22, 2010. Accepted June 23, 2010. This work was supported by the Australian Research Council (Grants LP0882775, FF0457721, and DP0880484) and the Centres of Excellence Program of the Government of Western Australia.