Organic &
Biomolecular
Chemistry

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 4069

www.rsc.org/obc

PAPER

Solar irradiation of the seed germination stimulant karrikinolide produces two novel head-to-head cage dimers†

Adrian Scaffidi,[a] Mark T. Waters,[a] Brian W. Skelton,[b] Charles S. Bond,[a] Alexandre N. Sobolev,[a] Rohan Bythell-Douglas,[a] Allan J. McKinley,[a] Kingsley W. Dixon,[c,d] Emilio L. Ghisalberti,[a] Steven M. Smith[a] and Gavin R. Flematti*[a]

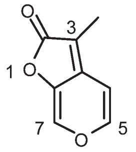
Received 12th January 2012, Accepted 26th March 2012
DOI: 10.1039/c2ob25090j

Karrikinolide is a naturally derived potent seed germination stimulant that is responsible for triggering the germination of numerous plant species from various habitats around the world. We now report that solar irradiation of karrikinolide yields two novel head-to-head cage photodimers with the formation, stability and bioactivity of both presented herein.

Introduction

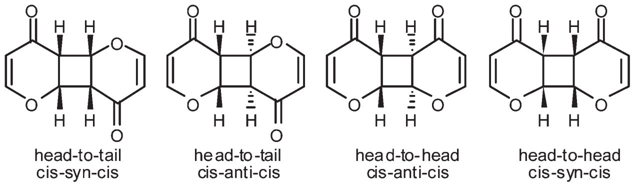
The discovery of the potent seed germination stimulant 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one **1** from smoke,[1] more commonly known as karrikinolide (Fig. 1), has generated significant research interest due to the diverse range of plant species present in both fire and non-fire prone regions that respond to this intriguing small molecule.[2–4] Several other compounds with similar structure to **1** have also been identified in smoke leading to the collective name 'karrikins'.[5] The broad species recognition and highly potent characteristics of **1** at concentrations as low as one part-per-billion,[1,6] provide a unique chemical tool for studying seed dormancy[7,8] and will enable significant cost benefits for plant restoration programs through enhanced seed germination and seedling establishment.[9–12] Therefore, understanding the environmental stability and fate of **1** is critical to implementing a successful transition from research to commercial applications. In an attempt to shed light on these challenges, we set out to investigate the environmental fate, and more specifically, the UV stability of the seed germination stimulant **1**.

It has previously been observed that similar compounds containing 4-pyrone rings can undergo various photochemical reactions.[13,14] One of the main processes is a [2 + 2] photocycloaddition to furnish cyclobutane photodimers of either head-to-tail or head-to-head orientation.[15–18] Furthermore, each photodimer can result in either *cis–syn–cis* or *cis–anti–cis* isomers making outcome prediction difficult (Fig. 2).



1

Fig. 1 Karrikinolide seed germination stimulant.



head-to-tail cis-syn-cis
head-to-tail cis-anti-cis
head-to-head cis-anti-cis
head-to-head cis-syn-cis

Fig. 2 General photodimer structures of 4-pyrones.

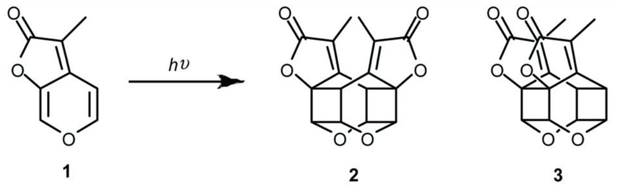
In addition, a limited number of cage photodimers resulting from a second cyclization of *cis–syn–cis* head-to-tail photodimers have also been reported,[17,19–21] however, to the best of our knowledge, no head-to-head cage photodimers of 4-pyrones have been unequivocally characterised.[20] Interestingly, formations of the head-to-tail cage photodimers *via* a double [2 + 2] cycloaddition have been shown to occur not only in solution but also in the solid state if the molecular arrangement of the monomer crystal permits.[18,22] More specifically, the centre-to-centre distances between the two reacting alkenyl groups must be within the limits proposed by Schmidt of 4.2 Å to initiate the [2 + 2] photocycloaddition.[23,24]

The solar stability of **1** was initially evaluated by irradiating a dilute solution with natural sunlight. The reaction was monitored

---

[a] *School of Chemistry and Biochemistry, The University of Western Australia 35 Stirling Highway, Crawley, 6009 WA, Australia. E-mail: gavin.flematti@uwa.edu.au*
[b] *Centre for Microscopy, Characterisation and Analysis, The University of Western Australia, 35 Stirling Highway, Crawley, 6009 WA, Australia*
[c] *School of Plant Biology, The University of Western Australia, 35 Stirling Highway, Crawley, 6009 WA, Australia*
[d] *Kings Park Botanic Garden, Fraser Avenue, West Perth, 6005 WA, Australia*

† Electronic supplementary information (ESI) available: NMR spectra. CCDC 837152 and 837151. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2ob25090j



1 **2** **3**

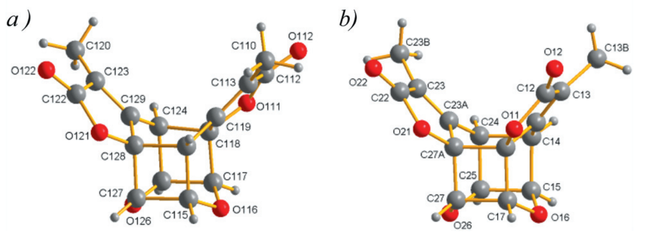


Fig. 3 Karrikinolide cage photodimers **2** and **3**. (a) Structure of molecule 1 of cage photodimer **2** (second molecule similar). (b) Structure of cage photodimer **3**. All atoms have been drawn with arbitrary radii.

by high performance liquid chromatography (HPLC) with the complete consumption of **1** observed within hours; however, it was not clear whether **1** simply degraded or if products resulted from a photochemical reaction. Further investigations by gas chromatography-mass spectrometry (GC-MS) indicated the presence of two dimeric species with nuclear magnetic resonance (NMR) spectroscopy confirming two cage photodimers due to the absence of any downfield signals.

Given the symmetry generated by the formation of cage photodimers, NMR spectroscopy does not discriminate between head-to-head and the corresponding head-to-tail cage structures. For example, head-to-head cage dimers with a mirror plane of symmetry cannot be distinguished from head-to-tail cage dimers with a two-fold axis of rotation. Likewise, head-to-head cage dimers with a two-fold axis of rotation cannot be distinguished from head-to-tail cage dimers with an inversion centre of symmetry. As a result, single crystal X-ray structure elucidation was required to unequivocally assign two novel karrikinolide head-to-head cage photodimers **2** and **3** (Fig. 3).

The unique cage photodimer **2** is a result of a double photocyclisation of two molecules of **1** superimposed with one molecule rotated 180° along the C(3)–O(6) line. In contrast, two superimposed molecules of **1** resulted in the cage photodimer **3**. The crystal structure confirms the geometry of cage photodimer **2** as having a pseudo two-fold axis relating the two butenolide rings in comparison with the pseudo-mirror symmetry in the structure of cage photodimer **3**. There are two independent molecules in the asymmetric unit of **2**. Due to the lack of precision of the determination, meaningful comparisons of the geometries with those of structure **3** were not possible. The angles between the two butenolide rings are 85.0(8) and 87.9(9)° for molecules 1 and 2 of cage photodimer **2** respectively, compared to 82.05(5)° for those of cage photodimer **3**.

To investigate further the degradation of **1** and the formation of cage photodimers **2** and **3** by means of solar radiation, a dilute solution of **1** was irradiated under controlled conditions employing a solar lamp simulator. This allowed the reaction to be monitored at constant power output and removed variations associated with natural solar UV indices. Once again, GC-MS indicated the presence of two cage photodimers formed within the first hour with the reaction requiring a full day of irradiation to see complete consumption of the starting material (Fig. 4). The formation of both cage photodimers reached a maximum around nine hours and were also found to degrade upon further exposure to solar radiation with no further products observed by GC-MS.

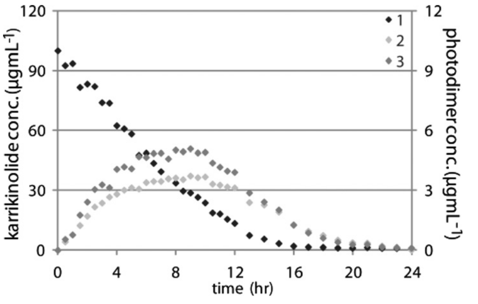


Fig. 4 The concentrations of **1** (primary axis), **2** and **3** (secondary axis) after irradiating a 100 μg mL^{-1} solution of **1**.

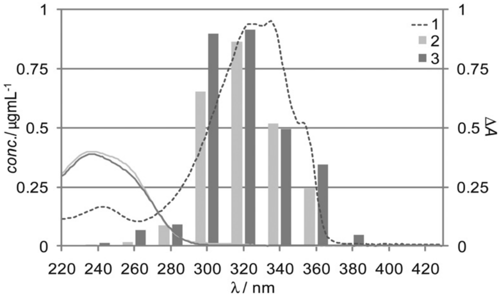


Fig. 5 Concentration of cage photodimer **2** and **3** (primary axis) produced at various wavelengths and UV absorbance spectrum of **1**, **2** and **3** (secondary axis).

We then turned our attention towards identifying the optimal wavelength to afford the cage photodimers by irradiating a solution of **1** with a high pressure xenon–mercury lamp fitted with a monochromator. The reaction was monitored by GC-MS and it was found that the optimal wavelength for cage photodimer formation was within the range of 300 nm to 320 nm, consistent with the UV absorbance of **1** (Fig. 5).

Having successfully isolated two head-to-head cage photodimers from irradiation of a dilute solution of **1**, we set about investigating whether similar transformations could occur in the solid state as previously reported for 4-pyrones.[18,22] To this end, a crystal of 1 was irradiated with the solar simulator for several hours but no dimerisation was observed by X-ray crystallography. This result was not surprising as although the centre-to-centre distances between the two reacting alkenyls have been determined to be within the limits proposed by Schmidt,[23,24] the orientations are such that one unit would need to rotate approximately 60° to orientate anti-parallel along the C(3)–O(6) line.[25]

During efforts to optimise GC-MS conditions for the detection of karrikinolide **1** and the two cage photodimers **2** and **3**, we observed that the amount of each compound detected had an optimal injection temperature (Fig. 6). Above this temperature, a reduced quantity of each compound was detected indicating that all three butenolides were sensitive to elevated temperatures.

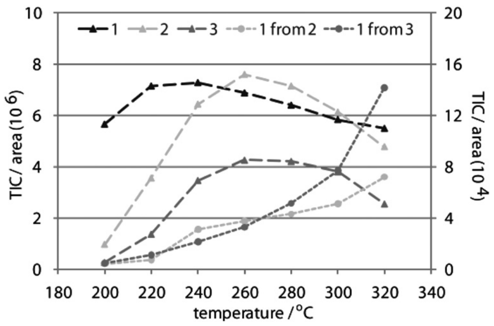


Fig. 6 Total-ion-count for a 10 µg mL^{-1} solution of **1**, **2** and **3** at various temperatures (primary axis), and total-ion-count for **1** produced from **2** and **3** at various temperatures (secondary axis).

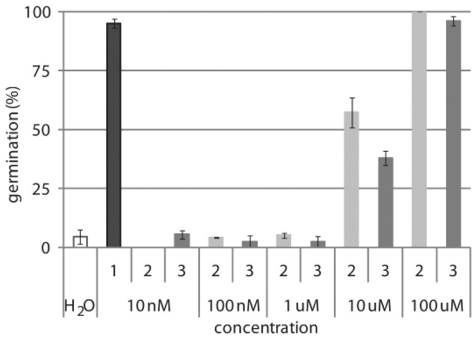


Fig. 7 Germination of *Solanum orbiculatum* seeds in response to cage photodimers **2** and **3**.

Moreover, a rise in injection temperature resulted in increased amounts of **1** detected from each of the cage photodimers indicating the thermal reversibility of the facile double photocyclisation reaction required to furnish **2** and **3**.

The reduction of **1** observed for injection temperatures above 240 °C is of particular interest given **1** is produced from burning plant material.[1] Since wildfire flame temperatures can reach several hundred degrees,[26] we speculate that **1** is mainly produced in the cooler parts of the fire and must be removed from the source of the fire in the smoke or condensate fraction allowing it to ultimately stimulate germination.

The two cage photodimers were purified by semi-preparative HPLC prior to screening for stimulatory activity against *Solanum orbiculatum*[11] seeds under a range of concentrations. Somewhat surprisingly, given recent structure–activity investigations suggest substitutions at C4 significantly reduce activity,[27] both photodimers appeared to promote germination above control levels at higher concentrations (Fig. 7). However, given that **1** is active below nanomolar concentrations[1] and given the reversibility of the cage photodimers at elevated temperatures, we cannot rule out the presence of trace amounts of **1** which may account for this unusual stimulatory ability.

Our results suggest that exposure to solar radiation after a wildfire may result in smoke-derived **1** being consumed within a relatively short period of time. However, soil exposed to plant-derived smoke has been shown to promote germination several years after a fire event[28] and we have detected the presence of **1** in soil collected several months after a wildfire. In contrast, we have not detected **2** or **3** in the same samples, which confirms that dimerization of **1** is restricted in the solid state. In addition, some of the 4800 compounds[29] produced during a fire may protect **1** from UV degradation, allowing it to remain in the environment for prolonged periods of time.

Conclusion

In summary, our study demonstrates that upon irradiation with a solar light source, the seed germination stimulant **1** affords two novel head-to-head cage photodimers **2** and **3**. In addition to reporting the first example of the unique cage moiety, it has been shown that the facile double photocycloaddition is reversible under elevated temperatures enabling the photodimers to revert back to the monomer unit. Moreover, we have highlighted the reactivity of karrikinolide **1** to solar radiation which suggests that suitable formulations containing UV stabilisers may be required to enable **1** to be used effectively in plant regeneration and land conservation programs.

Experimental

General

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on a Bruker AV600 (600 MHz for δH and 150.9 MHz for δC) spectrometer. Hexadeuteroacetone ($(CD_3)_2CO$) was used as the solvent with residual $(CH_3)_2CO$ (δH 2.05) or $(CD_3)_2CO$ (δC 29.84) being employed as internal standards. Melting points (m.p.) were determined on a Reichert hot stage melting point apparatus. High-resolution mass spectra (HR-MS) were recorded on a Waters GCT spectrometer using chemical ionisation (45 eV) (CI) technique. Gas chromatography-mass spectrometry (GC-MS) were recorded on an Agilent 5973 instrument using electron ionisation (EI) technique. UV-Vis data was collected on a Perkin-Elmer Lambda 25 spectrophotometer. Flash chromatography was performed on Merck silica gel 60 with the specified solvents and thin-layer chromatography (TLC) was effected on Merck silica gel 60 F254 aluminium-backed plates. Karrikinolide **1** was prepared as previously described.[30]

Cage photodimer isolation

In a silica glass reaction vessel was dissolved karrikinolide **1** (50 mg, 0.3 mmol) in a mixture of methanol and water (30 mL, 2 : 1) and the resulting solution irradiated for 24 h with an Oriel Instruments Research Arc Lamp (Newport: 66907) fitted with a xenon lamp (18.2 V, 5.5 A) and AM1.5G filter. The solution was concentrated under vacuum followed by flash chromatography (30%–70% ethyl acetate–hexanes) to furnish the cage photodimer **2** as colourless needles after recrystallisation (dichloromethane–hexanes) (6.0 mg, 12%), m.p. > 250 °C; ^{1}H NMR δ 5.05 (dd, ^{3}J(H5/5′,H7′/7) = 8.2 Hz and ^{3}J(H4/4′,H5/5′) = 8.1 Hz, 1H; H5/5′), 5.01 (dd, ^{4}J(H4′/4,H7/7′) = 2.46 Hz 1H; H7/7′), 4.52 (dd, 1H; H4/4′), 1.77 (s, 3H; H3b/3′b). ^{13}C NMR δ 173.21 (C2/2′), 154.18 (C3a/3′a), 121.59 (C3/3′), 85.51, 78.84, 68.24 (C5/5′, C7/7′, C7a/7′a), 44.68 (C4/4′), 8.54 (C3b/3′b). UV/Vis (methanol): $\lambda_{max}(\varepsilon)$ = 235 nm (12 180); CI-HRMS: *m*/*z* 301.0712, ([M + H]$^{+}$ $C_{16}H_{13}O_6$ requires 301.0717). EI-MS: *m*/*z* (%) 254(5), 150(25), 121(28), 84(100). Next to elute was the cage photodimer **3** yielding needles after recrystallisation (dichloromethane–hexanes) (6.0 mg, 12%), m.p. > 250 °C; ^{1}H NMR δ 5.10 (s, 1H; H7/7′), 4.95 (m, 1H; H5/5′), 4.47 (m, 1H; H4/4′), 1.74 (s, 3H; H3b/3′b). ^{13}C NMR δ 173.27 (C2/2′), 155.07 (C3a/3′a), 120.98 (C3/3′), 86.83, 76.05, 72.49 (C5/5′, C7/7′, C7a/7′a), 38.73 (C4/4′), 8.34 (C3b/3′b). UV/Vis (methanol): $\lambda_{max}(\varepsilon)$ = 235 nm (11 840); CI-HRMS: *m/z* 301.0705, ([M + H]$^+$ $C_{16}H_{13}O_6$ requires 301.0717). EI-MS: *m/z* (%) 300(M$^+$,7), 227(45), 216 (30), 150(41), 122(24), 121(90), 84(100), 65(24). Semi-preparative HPLC was conducted using a Hewlett Packard 1050 system equipped with a multiple wavelength detector (MWD). Separation was achieved using a 250 × 10 mm i.d., 5 µm, Apollo C18 reversed phase column (Grace-Davison) with a 33 mm × 7 mm guard column of the same material. The column was eluted at 4 mL min^{-1} with 30% (v/v) acetonitrile–water and 1 mL was injected. UV absorbance was monitored at wavelengths of 210, 254 and 325 nm. Photodimer **2** R_t = 23.67 min, photodimer **3** R_t = 24.48 min.

Wavelength optimisation

A solution of **1** (1.0 mL, 0.1 mg mL^{-1}) in methanol was irradiated for 6 h at different wavelengths (240–400 nm) using a high pressure mercury/xenon lamp (1000 W, Newport: 6293) fitted with a monochromator. The radiation intensity was measured at each wavelength with a laser power meter (Ophir AN/2) with all measurements normalized to the intensity at 300 nm. The resulting solutions were analysed by GC-MS using a Varian factor four column (VF-5 ms, 30 m × 0.25 mm i.d., 0.25 µm) with the initial oven temperature set at 50 °C and held for 1 min before increasing at 15 °C min^{-1} to 250 °C, then 5 °C min^{-1} to 300 °C, and held for 10 min (inlet temperature 260 °C; transfer line 280 °C). The ion source was set at 200 °C and the spectrometer was set to record in selective ion monitoring (SIM) mode using ions *m/z* 121 and 150 for **1** and *m/z* 84 and 121 for the cage photodimers **2** and **3**. Quantitation was achieved using *m/z* 121 for **1** and *m/z* 84 for both cage photodimers. R_t (photodimer **2**) = 19.98 min, R_t (photodimer **3**) = 22.37 min.

Structure determination

Crystallographic data for both compounds were collected at 100 K, the Beamline PX2 at the Australian Synchrotron[31] (λ = 0.71077 Å) being used for **2** and an Oxford Diffraction Gemini diffractometer fitted with Mo Kα radiation (λ = 0.71073 Å) for compound **3**. For both **2** and **3**, solution was by direct methods with the structures being refined against F2 with full-matrix least-squares using the program SHELXL-97.[32] All H-atoms were added at calculated positions and refined by use of a riding model with isotropic displacement parameters based on those of the parent atom.

Crystal data for **2**: Formula $C_{16}H_{13}O_6$, M_r = 300.26, monoclinic, *P*21/*c*, *a* = 17.0210(4) Å, *b* = 11.9940(10) Å, *c* = 13.4560 (5) Å, β = 113.283(4), *V* = 2523.3(2) Å^3, *Z* = 8, ρ_{calcd} = 1.581 g cm^{-3}, μ = 0.123 mm^{-1}, 2θ_{max} = 50°, 16 855 reflections collected, 4267 unique, (R_{int} = 0.182), R_1 = 0.266, wR_2 = 0.569. CCDC No. = 837152. The crystal was treated as a merohedral twin with approximately 50/50 occupancy. The geometries of the two independent molecules were restrained to be similar. All atoms were refined with isotropic displacement parameters after anisotropic refinement resulted in many atoms with unacceptable ellipsoids even with the application of reasonable restraints. Attempts to obtain a disordered model for either of the molecules or a superior solution in a space group of lower symmetry were not successful.

Crystal data for **3**: Formula $C_{16}H_{13}O_6$, M_r = 300.26, monoclinic, *P*21/*c*, *a* = 6.8238(2) Å, *b* = 11.0492(3) Å, *c* = 16.3885(4) Å, β = 100.760(2)°, *V* = 1213.93(6) Å^3, *Z* = 4, ρ_{calcd} = 1.643 g cm^{-3}, μ = 0.127 mm^{-1}, 2θ_{max} = 63°, 22 968 reflections collected, 4234 unique, R_{int} = 0.040, R_1 = 0.039, wR_2 = 0.106. CCDC No. = 837151. Anisotropic displacement parameters were employed for the non-hydrogen atoms.

Seed bioassay

Germination experiments were performed using *Solanum orbiculatum* seeds collected in the Shark Bay region (Western Australia) and stored at −80 °C until use. Assays were conducted using Millipore water obtained by filtration through a Milli-Q ultrapure water system (Millipore, Australia). 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 7 days at 20 (±1 °C) with all experiments conducted in triplicate.

The authors thank the Australian Research Council (LP0882775) for financial support and the Australian Synchrotron, Victoria, Australia for use of the PX2 beamline. We also thank Jeremiah Toster for use of the solar simulator and E.D. Goddard-Borger for helpful discussions.

Notes and references

1 G. R. Flematti, E. L. Ghisalberti, K. W. Dixon and R. D. Trengove, *Science*, 2004, **305**, 977.
2 S. D. S. Chiwocha, K. W. Dixon, G. R. Flematti, E. L. Ghisalberti, D. J. Merritt, D. C. Nelson, J. A. M. Riseborough, S. M. Smith and J. C. Stevens, *Plant Sci.*, 2009, **177**, 252.
3 K. W. Dixon, D. J. Merritt, G. R. Flematti and E. L. Ghisalberti, *Acta Hortic.*, 2009, **813**, 155.
4 M. E. Light, M. I. Daws and J. Van Staden, *S. Afr. J. Bot.*, 2009, **75**, 1.
5 G. R. Flematti, E. L. Ghisalberti, K. W. Dixon and R. D. Trengove, *J. Agric. Food Chem.*, 2009, **57**, 9475.
6 J. C. Stevens, D. J. Merritt, G. R. Flematti, E. L. Ghisalberti and K. W. Dixon, *Plant Soil*, 2007, **298**, 113.
7 D. C. Nelson, A. Scaffidi, E. A. Dun, M. T. Waters, G. R. Flematti, K. W. Dixon, C. A. Beveridge, E. L. Ghisalberti and S. M. Smith, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 8897.
8 A. Scaffidi, G. R. Flematti, D. C. Nelson, K. W. Dixon, S. M. Smith and E. L. Ghisalberti, *Tetrahedron*, 2011, **67**, 152.
9 L. E. Commander, D. J. Merritt, D. P. Rokich and K. W. Dixon, *Bot. J. Linn. Soc.*, 2009, **161**, 411.
10 L. E. Commander, D. J. Merritt, D. P. Rokich and K. W. Dixon, *J. Arid Environ.*, 2009, **73**, 617.
11 L. E. Commander, D. J. Merritt, D. P. Rokich, G. R. Flematti and K. W. Dixon, *Aust. J. Bot.*, 2008, **56**, 333.
12 D. J. Merritt, M. Kristiansen, G. R. Flematti, S. R. Turner, E. L. Ghisalberti, R. D. Trengove and K. W. Dixon, *Seed Sci. Res.*, 2006, **16**, 29.
13 A. R. Katritzky and N. Dennis, *Chem. Rev.*, 1989, **89**, 827.

14 Z. Stiplosek, M. Sindler-Kulyk, K. Jakopcic, A. Visnjevac and B. Kojic-Prodic, *J. Heterocycl. Chem.*, 2002, **39**, 37.

15 M. Sakamoto, M. Kanehiro, T. Mino and T. Fujita, *Chem. Commun.*, 2009, 2379.

16 M. Sakamoto, F. Yagishita, M. Kanehiro, Y. Kasashima, T. Mino and T. Fujita, *Org. Lett.*, 2010, **12**, 4435.

17 P. Yates, E. S. Hand, P. Singh, S. K. Roy and I. W. J. Still, *J. Org. Chem.*, 1969, **34**, 4046.

18 P. Yates and M. J. Jorgenson, *J. Am. Chem. Soc.*, 1963, **85**, 2956.

19 J. F. Eubank, V. Kravtsov and M. Eddaoudi, *J. Am. Chem. Soc.*, 2007, **129**, 5820.

20 D. Macgregor, *Mol. Photochem.*, 1974, **6**, 101.

21 P. Yates and M. J. Jorgenson, *J. Am. Chem. Soc.*, 1958, **80**, 6150.

22 H. Yan, H. Q. Wang, C. L. Ni and X. Q. Song, *Acta Crystallogr., Sect. E: Struct. Rep. Online*, 2006, **62**, o1951.

23 G. M. J. Schmidt, *Pure Appl. Chem.*, 1971, **27**, 647.

24 M. D. Cohen, *Angew. Chem., Int. Ed. Engl.*, 1975, **14**, 386.

25 G. R. Flematti, E. L. Ghisalberti, K. W. Dixon, R. D. Trengove, B. W. Skelton and A. H. White, *Aust. J. Chem.*, 2005, **58**, 505.

26 A. L. Sullivan, P. F. Ellis and I. K. Knight, *Int. J. Wildland Fire*, 2003, **12**, 101.

27 G. R. Flematti, A. Scaffidi, E. D. Goddard-Borger, C. H. Heath, D. C. Nelson, L. E. Commander, R. V. Stick, K. W. Dixon, S. M. Smith and E. L. Ghisalberti, *J. Agric. Food Chem.*, 2010, **58**, 8612.

28 I. T. Baldwin and C. A. Preston, *Ecology*, 1999, **80**, 481.

29 C. J. Smith, T. A. Perfetti, R. Garg and C. Hansch, *Food Chem. Toxicol.*, 2003, **41**, 807.

30 E. D. Goddard-Borger, E. L. Ghisalberti and R. V. Stick, *Eur. J. Org. Chem.*, 2007, 3925.

31 T. M. McPhillips, S. E. McPhillips, H. J. Chiu, A. E. Cohen, A. M. Deacon, P. J. Ellis, E. Garman, A. G. Gonzalez, N. K. Sauter, R. P. Phizackerly, S. M. Soltis and P. J. Kuhn, *J. Synchrotron Radiat.*, 2002, **9**, 401.

32 G. M. Sheldrick, *Acta Crystallogr., Sect. A: Found. Crystallogr.*, 2008, **64**, 112.