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Su C. Cho

University of Nebraska-Lincoln

Patrick Dussault

University of Nebraska-Lincoln, pdussault1@unl.edu

Amber D. Lisec

University of Nebraska-Lincoln


Ellen C. Jensen

University of Nebraska-Lincoln, ejensen@csbsju.edu

Kenneth W. Nickerson

University of Nebraska-Lincoln, knickerson1@unl.edu

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Metathesis-based synthesis of jasmonate and homojasmonate lactones, candidates for extracellular quorum sensing molecules in *Candida albicans*

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Su C. Cho,^a Patrick H. Dussault,^{*a} Amber D. Lisec,^b Ellen C. Jensen^{b†} and Kenneth W. Nickerson^{*b}

^a Department of Chemistry and ^b School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE 68588, USA

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Ring-closing metathesis of jasmonate esters is shown to provide a rapid entry to jasmonate lactones. The lactones were investigated as possible quorum sensing molecules for the fungi *Candida albicans*. Assays demonstrated no effect on fungal morphology at concentrations up to 96 μM .

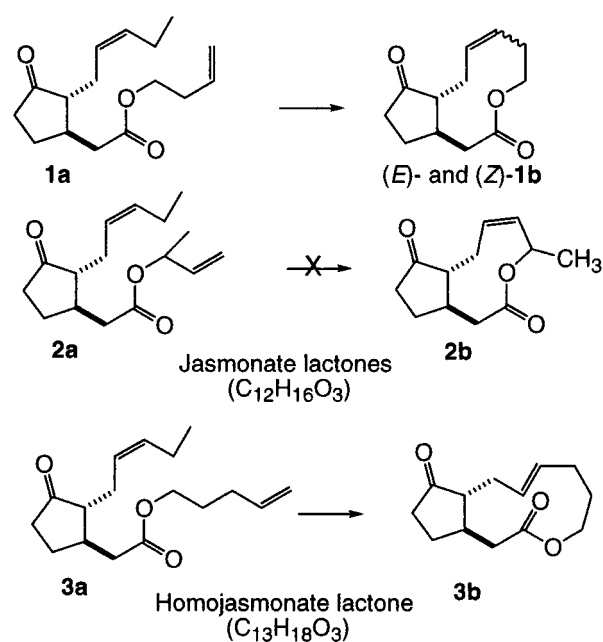
Introduction

We recently required a rapid synthetic approach to jasmonate lactones for screening as quorum-sensing molecules (QSMs) in fungi. An essential aspect of fungal pathogenicity is the capacity to undergo morphological interconversion. We have been studying an aspect of yeast-mycelial dimorphism termed the inoculum size effect in which inoculation above a given threshold level (usually 10^6 spores mL^{-1}) gives single celled, budding yeast while inoculation below that threshold level gives filamentous mycelial growth.¹ This effect is similar to quorum sensing in Gram negative bacteria, a phenomenon based on the excretion of acyl-homoserine lactones.² For both *Ceratocystis ulmi*, the causative agent of Dutch elm disease, and *Candida albicans*, a human pathogen,^{1,3} we have shown that the inoculum sensing mechanism relies on extracellular QSMs.^{3,4} For *C. albicans*, QSM activity could be extracted into ethyl acetate, resuspended in hexane or 90% methanol, and eluted from a C_8 reverse phase column with 90% methanol. Analysis of the active fraction by GC-MS showed two major peaks, one containing a molecular ion with a mass of 222 and a fragmentation pattern reminiscent of jasmonates. The possibility that the fungal QSMs were jasmonates was attractive because: (1) all fractions with QSM activity had a jasmonate-like aroma; (2) jasmonates are a new group of plant growth regulators which may act as positive transcription factors;^{5,6} and (3) jasmonates of unknown function have been discovered in the culture filtrates of other fungi.⁷ For example, 7 of 46 fungal strains studied by Miersch and co-workers were found to contain jasmonates, including jasmonic acid, isojasmonic acid and several hydroxylated jasmonic acids.⁷⁻¹⁰

The mass spectroscopic data, combined with the nonpolar nature of the unknown, led us to suspect a homologated jasmonate lactone as the putative QSM. In the course of preparing candidate structures, we discovered a remarkably rapid approach to jasmonate lactones based upon the use of ring-closing metathesis (RCM). We now report the synthesis of jasmonate lactones **1b** and **3b** and their bioassay for the ability to influence quorum sensing in *C. albicans* (Scheme 1).

Results and discussion

Given the limited structural information available at the inception of this project, synthetic elucidation of a candidate



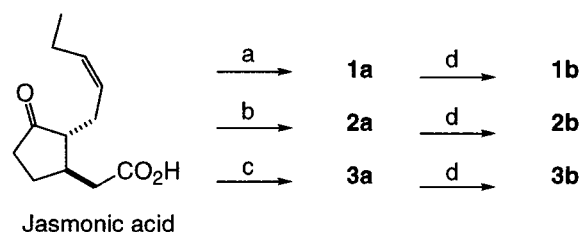
Scheme 1 Ring-closing metathesis approach to lactones.

structure threatened to require preparation of a large number of jasmonate lactones. Although 12-hydroxyjasmonate and the corresponding lactone (jasmine ketolactone) are known compounds,¹¹ the reported syntheses were either lengthy or were unsuitable for the synthesis of isomers or homologs.¹²⁻¹⁴ We therefore planned an approach based upon ring-closing metathesis (RCM) of jasmonate esters.^{15,16} As our initial targets for this approach, we investigated the preparation of the known jasmine ketolactone **1b** as well as two isomeric ketolactones, **2b** and **3b**. Lactone **2b** is a regioisomer of **1b** while **3b** is a homolog with the correct molecular mass for the candidate structure. As shown below, the RCM strategy would in principle allow synthesis of all three targets from readily obtained jasmonate esters.¹⁷

Synthesis

The starting materials for RCM, esters **1a**, **2a**, and **3a**, were readily prepared through esterification of racemic jasmonic acid with the appropriate unsaturated alcohol in the presence of dicyclohexylcarbodiimide (DCC). The individual esters were subjected to RCM in the presence of Grubb's ruthenium

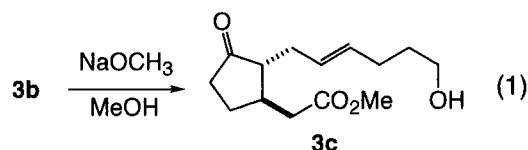
† Permanent address: Biological Sciences, St. John's University, Collegeville, MN, USA.



Scheme 2 Reagents: a. but-3-en-1-ol, DCC; b. but-3-en-2-ol, DCC; c. pent-4-en-1-ol, DCC; d. $(\text{C}_3\text{P})_2\text{Cl}_2\text{RuCHPh}$.

carbene under high dilution conditions (Scheme 2). Metathesis of **1a** afforded **1b** in moderate yield as a mixture of *E* and *Z* isomers which could be separated by preparative HPLC and identified in comparison with literature reports.¹⁷ The individual geometric isomers were each present as a single diastereomer. Metathesis of **2a** failed to provide isolable amounts of the corresponding lactone, an outcome presumably reflecting increased strain in the nine-membered ring lactone. Metathesis of **3a** proceeded in modest yield to furnish lactone **3b** as a single isomer.

Direct determination of the alkene geometry for **3b** was not feasible due to non-first order splitting in the ^1H NMR spectrum. However, the presence of an *E*-alkene could be demonstrated after cleavage of the lactone to the hydroxy methyl ester **3c** [eqn. (1)].



Comparison of synthetic materials with unknown QSM

As described in the Experimental section, the newly synthesized jasmonate lactones were compared by silica thin layer chromatography with methyl jasmonate and the unknown QSM. The R_f values were 0.54 for methyl jasmonate, 0.50 for the unknown QSM from *C. albicans*, 0.39 for lactone **3b**, and 0.36 for lactone **1b**. Thus, the putative QSM for *C. albicans* is more polar (lower R_f) than methyl jasmonate but less polar than either of the jasmonate lactones. The inability to detect any of the molecules with 254 nm UV light on fluorescent TLC plates indicated the absence of conjugated functional groups. Finally, the mass spectroscopic fragmentation patterns of the synthetic lactones differed significantly from that of the unknown QSM.

Assays for influence on quorum sensing

Lactones (**1a** and **3a**) were bioassayed for the ability to prevent germ tube formation (GTF) in *C. albicans*. However, no effect was observed at concentrations of up to 96 μM . In both assays, GTF was $\geq 90\%$ relative to assays conducted without the addition of lactone. For comparison, autoinducers of Gram negative bacteria such as 3-oxohexanoyl-L-homoserine lactone are active at nM concentrations, while those plants which respond to methyl jasmonate often do so at concentrations at or below 40 mM.^{2,5,6}

In conclusion, we have developed a new and rapid approach to the synthesis of jasmonate lactones. Further investigations into the identity of the fungal QSMs are in progress and will be reported in due course.

Experimental

General

All reagents and solvents were used as supplied commercially, except THF and CH_2Cl_2 , which were distilled from Na- Ph_2CO

and CaH_2 , respectively. ^1H and ^{13}C NMR spectra were recorded on G.E. Omega-300, or G.E.-500 MHz spectrometers in CDCl_3 ; individual peaks are reported as (multiplicity, number of hydrogens, coupling constant in Hz). Infrared spectra were recorded on an Analect RFX-65 FT-IR spectrophotometer as neat films unless otherwise stated. Selected absorbances are reported in wavenumbers (cm^{-1}). Elemental analyses were obtained from M-H-W Laboratories, Phoenix, AZ, USA, Desert Analytics, Tucson, AZ, USA, or Quantitative Technologies, Inc., NJ, USA. Mass spectra were obtained at the Nebraska Center for Mass Spectrometry. Much of the chromatography was performed with recycled ethyl acetate-hexane reformulated using a reported procedure.¹⁸

Isolation of the putative QSM and mass spectroscopic data

Candida albicans A-72 was grown in Fernbach flasks containing 1 L of glucose-phosphate-proline medium.¹ Flasks were inoculated at a cell density of 10^7 per mL and shaken for 24 h at 30 °C and 110 rpm. The cells were removed by centrifugation and the supernatants were sterilized by passage through 0.2 μm filters. Active supernatant (total 15.5 L) was extracted with equal volumes of ethyl acetate and the organic layer was concentrated by rotary evaporation. The dried residue was resuspended in 1.5 L of 90% MeOH and subdivided into 100 mL aliquots, which were individually extracted with hexane (2×70 mL). The pooled hexane fractions were concentrated by rotary evaporation and resuspended in 15.5 mL of 90% methanol. A 10.5 mL portion was loaded onto a C8 cartridge and eluted with 90% MeOH as 0.5 mL fractions, which were bioassayed for their ability to prevent germ tube formation. Active fractions were resuspended in 100% methanol and analyzed by GC-MS, (30 meter DB-5 column) using CI (methane) ionization. The peak group eluting at 9.87 to 9.90 minutes contained an apparent molecular ion $(\text{M}+\text{H})^+$ at m/z 223, with other significant ions at 207, 205, 191, 181, 151, 137, 123, 113, and 109.

But-3-en-1-yl ester of jasmonic acid, but-3-en-1-yl 1,2-*trans*-3-oxo-2-[(*Z*)-pent-2-enyl]cyclopentane-1-acetate (**1a**)

DCC (22 mmol, 4.54 g) was added slowly into a solution of jasmonic acid (18.3 mmol, 3.86 g) in CH_2Cl_2 (91.5 mL) under N_2 in a dry 250 mL flask in a water bath. After stirring for 20 minutes, in the course of which a white precipitate formed, but-3-en-1-ol (22 mmol, 1.9 mL) was added slowly with a syringe. DMAP (0.9 mmol, 0.111 g) was added to the solution which was allowed to stir for 3 days, whereupon TLC indicated completion of the reaction. Diethyl ether (50 mL) was added to the mixture to precipitate urea. The concentrated filtrate was purified by flash chromatography (10% ethyl acetate-hexane) to give 4.01 g (83%) of the ester as a yellow oil: $R_f = 0.43$ (10% ethyl acetate-hexane); ^1H NMR (500 MHz, CDCl_3) 5.74 (ddt, 1H, $J = 17.2, 10.4, 6.8$), 5.41 (m, 1H), 5.22 (m, 1H), 5.08 (dd, 1H, $J = 17.0, 1.6$), 5.04 (dd, 1H, $J = 10.4, 0.9$), 4.12 (t, 2H, $J = 6.6$), 2.66 (m, 1H), 2.38–2.17 (7H), 2.10–1.99 (4H), 1.85 (apparent dt, 1H, $J = 8.9, 5.6$), 1.46 (m, 1H), 0.92 (t, 3H, $J = 7.6$); ^{13}C NMR (125 MHz, CDCl_3) 218.7, 171.9, 134.0, 133.9, 124.9, 117.2, 63.45, 53.9, 38.9, 38.0, 37.6, 33.0, 27.1, 25.4, 20.5, 14.0; IR (Neat) 2962, 2933, 1746, 1653, 1644, 1465, 1233, 1166, 990, 633 cm^{-1} ; [HRMS(EI) Calcd. for $\text{C}_{16}\text{H}_{24}\text{O}_3$ (M)⁺: 264.1725. Found: 264.1724].

But-3-en-2-yl ester of jasmonic acid, but-3-en-2-yl 1,2-*trans*-3-oxo-2-[(*Z*)-pent-2-enyl]cyclopentane-1-acetate (**2a**)

Compound **2a** was prepared in 76% yield from but-3-en-2-ol by a similar procedure: $R_f = 0.44$ (10% ethyl acetate-hexane); ^1H NMR (500 MHz, CDCl_3) 5.80 (ddd, 1H, $J = 16.1, 10.5, 6.0$), 5.41 (m, 1H), 5.33 (m, 1H), 5.22 (m, 1H), 5.21 (dd, 1H, $J = 16.9, 1.2$), 5.11 (d, 1H, $J = 10.9$), 2.67 (m, 1H), 2.34–2.17 (5H), 2.10–1.98 (4H), 1.85 (apparent dt, 1H, $J = 10.1, 5.6$), 1.47 (m, 1H),

1.28 (d, 3H, $J = 6.5$), 0.91 (t, 3H, $J = 7.6$); ^{13}C NMR (125 MHz, CDCl_3) 219.59, 171.92, 138.18, 134.66, 125.60, 116.67, 71.81, 54.61, 39.92, 38.70, 38.35, 27.74, 26.06, 21.21, 20.55, 14.72; IR (Neat) 2967, 2936, 1744, 1463, 1425, 1377, 1233, 1168, 992, 670 cm^{-1} ; [HRMS(EI) Calcd. for $\text{C}_{16}\text{H}_{24}\text{O}_3$ (M) $^+$: 264.1725. Found: 264.1736].

Pent-4-enyl ester of jasmonic acid, pent-4-en-1-yl 1,2-*trans*-3-oxo-2-[(*Z*)-pent-2-enyl]cyclopentane-1-acetate (3a)

Compound **3a** was prepared in 77% yield by a similar procedure from pent-4-en-1-ol: $R_f = 0.37$ (10% ethyl acetate–hexane); ^1H NMR (500 MHz, CDCl_3) 5.79 (ddt, 1H, $J = 17.3, 10.5, 6.8$), 5.44 (m, 1H), 5.25 (m, 1H), 5.02 (dd, 1H, $J = 16.9, 1.6$), 4.98 (dd, 1H, $J = 10.1, 1.2$), 4.09 (t, 2H, $J = 6.8$), 2.68 (m, 1H), 2.39–2.19 (5H), 2.13–2.01 (6H), 1.87 (apparent dt, 1H, $J = 8.9, 5.6$), 1.73 (quintet, 2H, $J = 6.9$), 1.49 (m, 1H), 0.94 (t, 3H, $J = 7.6$); ^{13}C NMR (125 MHz, CDCl_3) 218.4, 171.8, 137.1, 133.8, 124.8, 115.1, 63.7, 53.8, 38.8, 37.9, 37.5, 29.8, 27.6, 27.0, 25.3, 20.4, 13.9; IR (Neat) 2962, 2935, 1745, 1653, 1642, 1464, 1232, 1167, 994, 604 cm^{-1} ; [HRMS(EI) Calcd. for $\text{C}_{17}\text{H}_{26}\text{O}_3$ (M) $^+$: 278.1882. Found: 278.1882].

Ring-closing metathesis

Jasmine ketolactone [(*E* and *Z*)-1,2-*trans*-8,13-dioxo-7-oxa-bicyclo[8.3.0]tridec-3-ene] (**1b**). Into a dry 1000 mL three-neck round bottom flask under N_2 containing CH_2Cl_2 (200 mL) was added a solution of bis(tricyclohexylphosphine)benzylidene ruthenium(IV) dichloride (0.34 mmol, 0.280 g) in CH_2Cl_2 (200 mL) from a pressure equalizing dropping funnel. A solution of ester **1a** (3.4 mmol, 0.900 g) in CH_2Cl_2 (200 mL) was added by a syringe pump over ~12 h. The resulting mixture was refluxed for 2 days, whereupon more ruthenium catalyst (0.1 mmol, 0.08 mg) was slowly added in a solution of CH_2Cl_2 (50 mL). The reaction was refluxed for ~24 h and then concentrated *in vacuo*. The residue was filtered through a short column of silica with 10–25% ethyl acetate–hexane and the concentrated filtrate was subjected to flash chromatography (5–25% ethyl acetate–hexane) to furnish 0.395 g (59%) of jasmine ketolactone as a 2:1 *E*–*Z* mixture which could be separated by semi-preparative HPLC (5% propan-2-ol–hexane, Rainin Dynamax 8 μm Si): $R_f = 0.21$ (25% ethyl acetate–hexane); ^1H NMR (300 MHz, CDCl_3) 5.38 (m, 0.66H), 5.12 (m, 1.3H), 4.74 (td, 0.66H, $J = 10.7, 4.7$), 4.58 (dt, 0.33H, $J = 10.7, 4.6$), 3.81 (m, 0.66H), 3.73 (td, 0.33H, $J = 10.2, 3.5$), 2.78 (d, 0.66H, $J = 10.7$), 2.70 (dd, 0.33H, $J = 14.1, 2.8$), 2.61 (m, 0.33H), 2.52 (d, 0.66H, $J = 13.6$), 2.47–1.98 (7.4H), 1.89–1.64 (1.7H), 1.53–1.17 (0.9H); ^{13}C NMR (75 MHz, CDCl_3) 218.09, 217.67, 173.79, 171.92, 133.72, 130.99, 127.85, 125.89, 62.68, 62.06, 57.22, 55.80, 41.80, 41.18, 40.98, 38.91, 37.20, 37.09, 34.83, 33.91, 32.74, 28.92, 27.71, 25.54; IR (Neat) (*E*) 2980, 2958, 2908, 1744, 1685, 1288, 1160, 1136, 982, 742; (*Z*) 3056, 2978, 2857, 1738, 1657, 1286, 1186, 1138, 741, 721, 698, 661 cm^{-1} ; [HRMS(EI) Calcd. for $\text{C}_{12}\text{H}_{16}\text{O}_3$ (M) $^+$: 208.1099. Found: 208.1104]. Other peaks at m/z 191, 178, 163, 149, 138, 134, 122.

Homojasmonate lactone (*E*)-1,2-*trans*-9,14-dioxo-8-oxa-bicyclo[9.3.0]tetradec-3-ene (**3b**). The homojasmonate lactone was prepared from the pentenyl jasmonate **3a** by a similar procedure as employed for **1b** except that the reaction was refluxed for three days after the initial addition and then for four more days following recharge with additional catalyst. Purification as before furnished 0.054 g (28%) of a yellow oil as a single alkene isomer: $R_f = 0.22$ (25% EtOAc–hexanes); ^1H NMR (500 MHz, CDCl_3) 5.41 (m, 1H), 5.31 (m, 1H), 4.48 (td, 1H, $J = 10.9, 2.4$), 4.07 (dt, 1H, $J = 11.3, 3.6$), 2.58 (d, 1H, $J = 11.3$), 2.48 (dd, 1H, $J = 13.3, 2.41$), 2.45–1.42 (12 H); ^{13}C NMR (125 MHz, CDCl_3) 219.3, 172.0, 133.1, 126.8, 65.0, 54.4, 42.5, 38.7, 37.5, 34.7, 31.4, 28.6, 26.8; IR (Neat) 2948, 2918, 2847, 1751, 1685, 1676, 1653, 1276, 1161, 1069, 974, 740 cm^{-1} ; [HRMS(EI) Calcd. for

$\text{C}_{13}\text{H}_{18}\text{O}_3$ (M) $^+$: 222.1256. Found: 222.1257]. Other peaks at m/z 204, 179, 163, 135, 121.

Saponification of homojasmonate lactone {*trans*-3-oxo-2-[(*Z*)-6-hydroxyhex-2-enyl]cyclopentane-1-acetic acid, methyl ester} (**3c**). Into a solution of sodium methoxide (0.012 g) in MeOH (5 mL) under N_2 was added homojasmonate lactone **3b** (0.009 g, 0.04 mmole) as a solution in MeOH (0.5 mL). The reaction was allowed to stir for five days, whereupon TLC showed the reaction to be complete. The mixture was then extracted with 10% HCl (2 mL), water (10 mL), and 25% ethyl acetate–hexane (3×10 mL), dried with anhydrous Na_2SO_4 , and the solvent was evaporated under vacuum to yield (0.010 g, ~100% crude yield) of *trans*-3-oxo-2-[(*Z*)-6-hydroxyhex-2-enyl]cyclopentane-1-acetic acid, methyl ester (**3c**) which was used without further purification: ^1H NMR (500 MHz) 5.50 (dt, 1H, $J = 15, 7$), 5.35 (dt, 1H, $J = 15, 7$), 3.70 (s, 3H), 3.63 (t, 2H, $J = 6.4$), 2.62–2.68 (m, 1H), 2.39–2.20 (m, 6H), 2.12–2.06 (m, 3H), 1.87 (dt, 1H, $J = 9.7, 5$), 1.62 (dt, 2H, $J = 14.5, 7$), 1.54–1.47 (m, 1H); ^{13}C NMR (125 MHz, CDCl_3) 218.7, 172.6, 132.8, 126.8, 62.3, 54.0, 51.6, 38.7, 37.7, 37.6, 32.2, 30.9, 28.8, 27.2.

Chromatographic comparison with unknown QSM

TLC was performed on 250 μm Analtech GHLF silica plates. Samples were dissolved in 100% methanol prior to application (6–10 μL). TLC plates were developed in hexane–ethyl acetate (3:1) and the bands were visualized by dipping in a 1% aqueous potassium permanganate solution.

C. albicans bioassay

Washed *C. albicans* A72 cells stored at 4 °C in 50 mM potassium phosphate (pH 6.5) at a cell density of 2×10^9 mL^{-1} were tested for germ tube forming ability by inoculating them at a final concentration of 10^7 mL^{-1} into a prewarmed (37 °C) medium consisting of: 2.5 mM *N*-acetyl glucosamine, 3 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 10 mM *L*-proline, 111 mM glucose, and 50 mM potassium phosphate (pH 6.5). The cell suspensions (5 mL) were incubated for 4 hours at 37 °C in 25 mL Erlenmeyer flasks on a New Brunswick Scientific G-2 shaker at 175 rpm. After 4 hours the control cultures with no added QSMs gave ca. 90% GTF whereas those with active QSMs (such as Fraction 4) gave $\geq 90\%$ budding yeasts (0–10% GTF).

Acknowledgements

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References

- 1 R. K. Kulkarni and K. W. Nickerson, *Exp. Mycol.*, 1981, **5**, 148.
- 2 W. C. Fuqua, S. C. Winans and E. P. Greenberg, *J. Bacteriol.*, 1994, **176**, 269.
- 3 A. D. Lisee, MSc Thesis, University of Nebraska-Lincoln, 1998.
- 4 D. J. McNeel, MSc Thesis, University of Nebraska-Lincoln, 1983.
- 5 G. Sembdner and B. Parthier, *Plant Mol. Biol.*, 1993, **44**, 569.
- 6 P. E. Staswick, *Plant Physiol.*, 1992, **99**, 804.
- 7 O. Miersch, A. Preiss, G. Sembdner and K. Schreiber, *Phytochemistry*, 1987, **26**, 1037.
- 8 O. Miersch, J. Schmidt and G. Sembdner, *Phytochemistry*, 1989, **28**, 1303.
- 9 O. Miersch, G. Schneider and G. Sembdner, *Phytochemistry*, 1991, **30**, 4049.
- 10 O. Miersch, T. Gunther, W. Fritsche and G. Sembdner, *Nat. Prod. Lett.*, 1993, **2**, 293.

- 11 E. Demole, B. Wilhelm and M. Stoll, *Helv. Chim. Acta*, 1964, **47**, 1152.
- 12 P. Kuenzler and H. Gerlach, *Helv. Chim. Acta*, 1978, **61**, 2503.
- 13 T. Nishi and T. Kitahara, *Proc. Jpn. Acad., Ser. B.*, 1995, **71**, 20.
- 14 I. Shimizu and H. Nakagawa, *Tetrahedron Lett.*, 1992, **34**, 4957.
- 15 R. H. Grubbs, S. J. Miller and G. C. Fu, *Acc. Chem. Res.*, 1995, **28**, 446.
- 16 S. J. Armstrong, *J. Chem. Soc., Perkin Trans. 1*, 1998, 371.
- 17 During the course of our investigations, a synthesis of ketolactone **1b** was reported based upon a different ring-closing metathesis strategy: A. Fürstner and T. Müller, *Synlett*, 1997, 1010.
- 18 P. Dussault and K. Woller, *The Chemical Educator*, 1996, **1**, 1.

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