

ORIGINAL ARTICLE

Khatmiamycin, a motility inhibitor and zoosporicide against the grapevine downy mildew pathogen *Plasmopara viticola* from *Streptomyces* sp. ANK313

Muna Ali Abdalla¹, Hnin Yu Win¹, Md. Tofazzal Islam^{2,3}, Andreas von Tiedemann³, Anja Schüffler⁴ and Hartmut Laatsch¹

In the course of our screening for anti-peronosporomycetal agents, we isolated a new compound khatmiamycin (1), together with five known metabolites, GTRI-02 (3), 4-ethyl-5-methyl-heptanamide (4), aloesaponarin II (5), LL-C10037 α (6) and LL-C10037 β (7) from the culture broth of a terrestrial *Streptomyces* sp. ANK313. The structures of these metabolites were assigned on the basis of their spectroscopic data. Khatmiamycin (1) exhibited potent motility inhibitory (100%) and lytic ($83 \pm 7\%$) activities against zoospores of the grapevine downy mildew pathogen *Plasmopara viticola* at $10 \mu\text{g ml}^{-1}$, followed by compounds 5 (MIC $25 \mu\text{g ml}^{-1}$), 7, 6, 3 in the order of decreasing activity. Khatmiamycin (1) also showed potent antibacterial activity against *Staphylococcus aureus* and *Streptomyces viridochromogenes* (Tü57) by causing inhibition zones of 11 and 14 mm diameter, respectively, at the dose of $40 \mu\text{g}$ per disk. This is the first report on motility inhibitory and lytic activities of metabolites from a terrestrial *Streptomyces* species against the zoospores of downy mildew pathogen *P. viticola*.

The Journal of Antibiotics (2011) 64, 655–659; doi:10.1038/ja.2011.68; published online 3 August 2011

Keywords: antibacterial; naphthoquinones; oomycetes; *Streptomyces*; zoosporicide

INTRODUCTION

Peronosporomycetes are distinct from fungi and are phylogenetic relatives of brown algae and diatoms. They cause many destructive diseases in plants, animals, fishes and humans.^{1,2} One of the most notorious members of peronosporomycetes, *Plasmopara viticola*, is an obligate biotroph and a serious pathogen of grapevine worldwide. Many fungicides are ineffective against this phytopathogen, and hence, bioactive compounds with new modes of action are needed to combat this economically important pest. Under favorable environmental conditions, the fungus-like stramenopile, *P. viticola* infects grapevine leaves through characteristic biflagellate motile zoospores released from airborne sporangia coming from other infected plants. The zoospores aggregate to stomata of the grapevine leaf by swimming through water film and then rapidly encyst to become round cystospores by shedding their flagella.^{3,4} The cystospores then rapidly germinate to form germ tubes and penetrate host tissue through the stomata. Disruption of any of these asexual stages eliminates the potential for pathogenesis.⁵ The success of any zoosporic pathogen can be attributed in part to the speed of asexual differentiation to generate bi-flagellated motile zoospores and their ability to find a host through chemotaxis.⁶ Therefore, compounds that can interfere with normal swimming behavior and early development of *P. viticola* are supposed

to be important as lead compounds in the management of this notorious phytopathogen.⁴

Streptomycetes are known to produce diverse groups of interesting bioactive secondary metabolites.⁷ In the course of screening for secondary metabolites from actinomycetes, we found that the crude extract of a terrestrial *Streptomyces* sp. ANK313 remarkably inhibited motility of *P. viticola* zoospores and caused subsequent lysis at $100 \mu\text{g ml}^{-1}$. This observation prompted us to isolate and characterize the motility-inhibitory and lytic factors in the extracts by chromatographic fractionation. We have discovered a new motility inhibitor and zoosporicide named khatmiamycin (1) along with five known compounds from extracts of the culture broth of *Streptomyces* sp. ANK313. This study describes the isolation, structure elucidation and biological activity of khatmiamycin (1) and further known compounds towards zoospores of *Plasmopara viticola*.

MATERIALS AND METHODS

Optical rotation was measured on a Perkin-Elmer polarimeter (model 241) (Perkin-Elmer, San Jose, CA, USA). – NMR spectra were measured on a Varian Inova 600 (599.740 MHz) and a Varian Unity 300 (300.145 MHz) spectrometer. – ESI mass spectra were recorded on a Quattro Triple Quadrupole mass spectrometer with a Finnigan TSQ 7000 with nano-ESI API ion source. –

¹Institute of Organic and Biomolecular Chemistry, Georg-August University Göttingen, Göttingen, Germany; ²Department of Biotechnology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh; ³Department of Crop Sciences, Division of Plant Pathology and Plant Protection, Georg-August-University Göttingen, Göttingen, Germany and ⁴Institute of Biotechnology and Drug Research (IBWF), Kaiserslautern, Germany
Correspondence: Professor H Laatsch, Institute of Organic and Biomolecular Chemistry, University of Goettingen, Tammannstrasse 2, Goettingen D-37077, Germany.
E-mail: hlaatsc@gwdg.de

Received 15 December 2010; revised 28 June 2011; accepted 29 June 2011; published online 3 August 2011

HRESI mass spectra were measured on a Micromass LCT mass spectrometer coupled with a HP 1100 HPLC and a diode array detector. Column chromatography was carried out on MN silica gel 60, 0.05–0.2 mm; TLC was performed on Polygram SIL G/UV₂₅₄. All silica gel materials were purchased from Macherey-Nagel, Düren, Germany. – Size exclusion chromatography was done on Sephadex LH-20 (Lipophilic Sephadex; Amersham Biosciences, Freiburg, Germany, purchased from Sigma-Aldrich Chemie, Steinheim, Germany). XAD-16 adsorber resin was obtained from Rohm and Haas (Frankfurt, Germany).

Taxonomy of the producing strain

Streptomyces sp. ANK313 has been derived from a soil sample (Kaiserslautern) and was isolated on YMG agar at room temperature (YMG agar: 2 g l⁻¹ yeast extract, 5 g l⁻¹ malt extract, 5 g l⁻¹ glucose, 15 g l⁻¹ agar, 30 mg l⁻¹ cycloheximide). Its 16 S rRNA gene sequence was deposited in GenBank with the Accession Nr. HQ662219. The sequence shows high similarities to *Streptomyces ansochromogenes* subsp. *ansochromogenes* strain NBRC 13665 with 99.7% (GenBank Accession Nr. AB184448), and to *Streptomyces achromogenes* subsp. *streptozoticus* strain NBRC 14001 (GenBank Accession Nr. AB184562), as well as to *Streptomyces nogalater* strain NBRC 13445 (GenBank Accession Nr. AB184408), both with 99.6% homology. The strain *Streptomyces* sp. ANK313 is deposited in the culture collection at the Institute of Organic and Biomolecular Chemistry, Göttingen, Germany.

Fermentation, extraction and isolation

The *Streptomyces* sp. ANK313 was cultivated on M₂⁺ medium for 7 days and extracted with ethyl acetate (mycelium) and XAD-16 resin (filtrate), respectively, as described for related strains.⁸ The combined extracts were chromatographed on silica gel using a CH₂Cl₂/MeOH gradient (0–10% MeOH) to obtain three fractions (Figure 1). On TLC, fraction II showed two yellow components, which were further purified on Sephadex LH-20 followed by PTLC and again Sephadex LH-20 to get three compounds **1** (2.6 mg), **3** (5.8 mg) and **4** (7.0 mg). Fraction III was subjected to Sephadex LH-20 using MeOH followed by RP-18 to deliver compounds **5** (2.1 mg) and **6** (19.5 mg). Purification of fraction IV on RP18 followed by PTLC revealed finally compound **7** (9.9 mg); for structures, see Figure 2).

Production of zoospores and bioassay

Sporangia of *P. viticola* were isolated from the infected leaves of grapevine (*Vitis vinifera* cv. Müller-Thurgau) stalk received from Prof Beate Berkelmann, Geisenheim Research Center, Germany. This strain was originally gained from

infected leaf materials of the grapevine cv. Riesling in 1996 and since then always propagated on fresh leaves of Müller-Thurgau. The strain was maintained by regular culturing on the lower surface of young grapevine leaves on Petri dishes containing 1.5% agar at 25 °C and 95% relative humidity.⁴ At day 6 of cultivation, the sporangiophores bearing lemon-shaped sporangia were harvested into an Eppendorf vial by a micro-vacuum cleaner.⁴ The freshly harvested sporangia were separated from sporangiophores by filtration through a nylon sieve (50 µm mesh), washed twice with distilled water and then incubated in sterilized tap water (3 × 10⁴ sporangia per ml) in the dark for 6 h at room temperature (23 °C) to release zoospores. These zoospores remained motile for 10–12 h in sterilized water and were used for the bioassay. Stock solutions of crude extracts and pure compounds were first prepared in

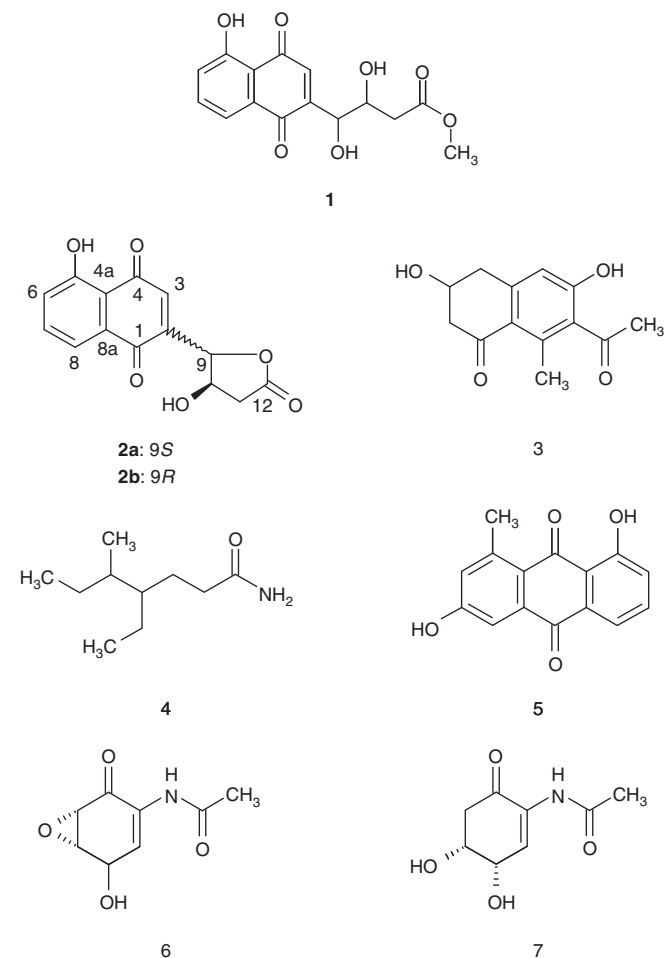


Figure 2 Structures of khatmiamycin (**1**), juglomycins A and B (**2a/b**) and other metabolites from *Streptomyces* sp. ANK313.

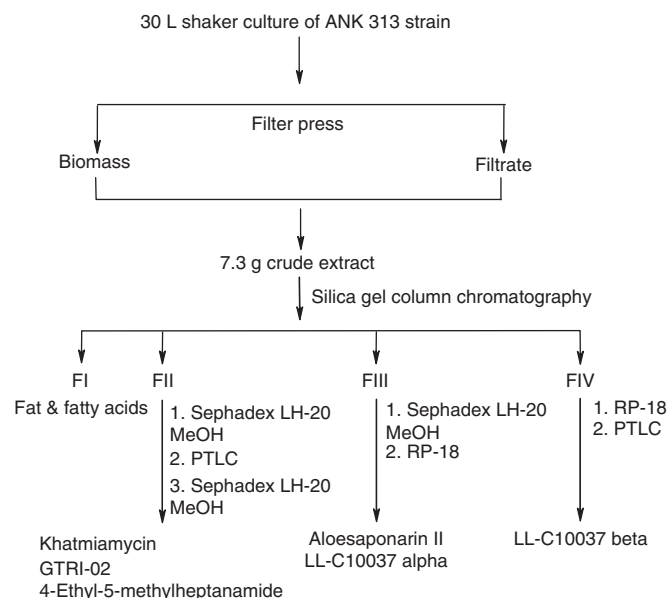


Figure 1 Isolation of khatmiamycin (**1**) and other metabolites.

Table 1 Physico-chemical properties of **1**

Appearance	Yellow solid
Molecular formula	C ₁₅ H ₁₄ O ₇
(+)-ESI MS (<i>m/z</i>)	329 (M+Na) ⁺
<i>R_f</i> value on TLC	0.49 ^a
[α] _D ²⁰	–21° (c 0.1, CH ₃ OH)
UV/VIS: λ _{max} (log ε), (MeOH)	243 (4.11), 265 (4.02), 322 (3.51), 412 (3.38)
Solubility	CH ₂ Cl ₂ , MeOH, DMSO

^aCH₂Cl₂/MeOH (95:5).

Table 2 ^{13}C (125 MHz) and ^1H (300 MHz) NMR data of khatmiamycin (**1**) in CD_2Cl_2 ; weak HMBC correlations are included in brackets

No.	δ_{C}	δ_{H} (mult.; J in Hz)	Selected HMBC correlations
1	184.5		
2	150.9		
3	135.4	7.04 (d, $J=1.4$)	C-1, C-2, C-4a, (C-5), C-9
4	190.3		
4a	115.2		
5	161.5		
6	124.6	7.27 (dd, $J=7.7, 1.9$)	C-4a, C-8
7	136.6	7.64 (t, $J=7.9$)	C-5, C-8a
8	119.3	7.62 (dd, $J=7.9, 2.5$)	C-1, C-4a, C-6
8a	132.4		
9	70.6	4.79 (s, br)	(C-2), C-3
10	69.6	4.21 (m)	
11	38.6	2.78 (ABX, $J=16.6, 8.4,$) 2.66 (1H, ABX, $J=16.6, 3.9,$)	C-10, C-12
12	173.1		
OMe	52.3	3.69 (s)	(C-11), C-12,
OH	—	11.82 (s)	C-4a, 5, 6, (7)

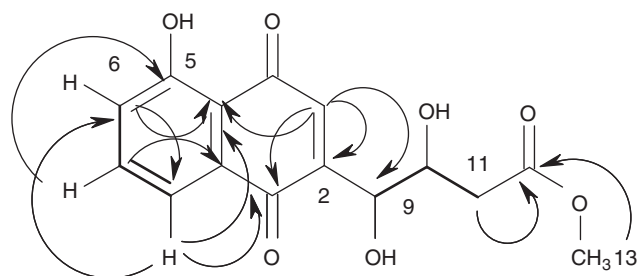
dimethyl sulfoxide (DMSO) and then diluted with distilled water. The concentration of DMSO in the zoospore suspension never exceeded 1% (v/v), a condition that does not affect zoospore motility. The bioassay was carried out as described earlier.^{5,9} Briefly, 40 μl of sample suspension was directly added to 360 μl of zoospore suspension (ca. 10^5 per ml) taken in a dish of plant tissue culture multi-well plate to make a final volume of 400 μl and then quickly mixed with a glass rod; 1% aqueous DMSO was used as a control. The motility of zoospores was observed under a light microscope at 10-fold magnification. Quantification of time-course changes of motility and lysis of zoospores were carried out as described earlier.⁴ Each treatment was replicated thrice. The mean value (%) \pm s.e. (standard error) of the affected spores in each treatment was calculated.

RESULTS AND DISCUSSION

Compound **1** was isolated as yellow solid (Table 1); it showed UV absorption at 254 nm, turned to black with anisaldehyde/sulfuric acid and became blue with 2N NaOH on TLC, indicating a peri-hydroxyquinone.

The ^1H NMR spectrum of **1** displayed an aromatic ABC system for three adjacent protons (see Supplementary Figure S1). In addition, there was a further narrow doublet at δ 7.04 ($J=1.4\text{ Hz}$). In the aliphatic region, the spectrum showed two oxygen-bound protons by signals at δ 4.79 and 4.21, a methoxy signal at δ 3.69 and diastereotopic methylene protons at δ 2.78 and 2.66. In the ^{13}C NMR (see Supplementary Figure S2) and HSQC spectra, there were 15 carbon signals as indicated by the HR ESIMS-derived formula $\text{C}_{15}\text{H}_{14}\text{O}_7$. Two ketone carbonyl groups at δ 190.3 and 184.5, one carbonyl of an acid or ester at δ 173.1, eight sp^2 carbons (four quaternary and four methine carbons) and four sp^3 carbons (two oxygenated CH, one OCH_3 and one CH_2) completed the spectrum (Table 2).

The HMBC spectrum showed correlations from H-7 to C-5 and C-8a, the former one (C-5) could be linked to oxygen, from H-8 to C-1 (184.5, CO), C-6 and C-4a, from H-6 to C-8 and C-4a. Furthermore, H-3 showed couplings with C-1 (CO), C-2, C-4a and C-9 of fragment II. H-11 and the methoxy group showed correlations with the carbonyl of an ester at δ 173.1 (C-12). According to the above spectroscopic data, a partial structure (Figure 3) with seven of the nine double-bond equivalents can be drawn.

**Figure 3** Selected H,H COSY (bold bonds) and HMBC correlations (arrows) of khatmiamycin (**1**).

The remaining ketone carbonyl at δ 190.3 showed no correlations. Placing this carbonyl at C-4 and closing the ring for the remaining double-bond equivalent completes structure **1**.

Numerous quinone derivatives have been isolated from *Streptomyces* spp. and fungi. Most heptaketides among them are having an additional annellated ring, forming anthraquinones or naphthopyranequinones, whereas compound **1** bears an open chain. Closely related with **1** are the juglomycins¹⁰ **A** (**2a**) and **B** (**2b**) and the juglone derivative NHAB.¹¹

Five further metabolites were identified as GTRI-02 (**3**),¹² 4-ethyl-5-methyl-heptanamide (**4**),¹³ aloesaponarin II (**5**),¹⁴ LL-C10037 α (**6**),¹⁵ and LL-C10037 β (**7**)¹⁶ by means of AntiBase and comparison of their data with the literature.

Motility inhibitory and lytic activities of khatmiamycin and other metabolites against *P. viticola* zoospores

The motility inhibitory and zoosporicidal activities of khatmiamycin (**1**) and the other metabolites of *Streptomyces* sp. ANK313 against downy mildew pathogen *P. viticola* are summarized in Table 3 and Figure 4. The motility of *P. viticola* zoospores was remarkably inhibited by **1** and **3–7** at low doses. Among the tested compounds, the highest motility inhibitory and lytic activities were recorded for compound **1** (MIC, $10\ \mu\text{g ml}^{-1}$) followed by compound **5** (MIC, $25\ \mu\text{g ml}^{-1}$). Other three compounds (**3**, **6** and **7**) showed almost equal strength of motility inhibitory and lytic activities against zoospores at $50\ \mu\text{g ml}^{-1}$.

Microscopic observation revealed that the crude extract and isolated metabolites showed zoosporicidal activity against *P. viticola* almost in a similar manner: initially, the zoospores were halted and the cellular materials gradually fragmented and dispersed into the surrounding water medium by bursting cell membranes. Motility inhibition and subsequent lysis of zoospores of the sugar beet damping-off pathogen *Aphanomyces cochlioides* by various kinds of natural products such as polyflavonoid tannins from the bark of *Lannea coromandelica*,⁹ anacardic acids from *Ginkgo biloba*,¹⁷ saponins from *Panax notoginseng* and macrocyclic lactam antibiotics from *Lysobacter* sp. SB-K88⁵ have been published. This is the first report on motility inhibition and lysis of zoospores of the downy mildew pathogen *P. viticola* by secondary metabolites from a terrestrial *Streptomyces* species. Motility of zoospores has been found critical for disease cycle and also virulence of the peronosporomycete phytopathogens.^{3,18} Our observation indicated that *Streptomyces* spp. produce potent new secondary metabolites, which might be useful for biorational and sustainable management of the peronosporomycete phytopathogens. Therefore, further studies on isolation of more zoospore regulating principles from other *Streptomyces* spp. may result in interesting metabolites or lead compounds with novel modes of action for developing

Table 3 Motility inhibitory and lytic activities of compounds isolated from *Streptomyces* sp. ANK 313 against the zoospores of grapevine downy mildew pathogen *Plasmopara viticola*

		Motility inhibitory and lytic activity (% ± s.e.) ^a against <i>Plasmopara viticola</i> zoospores							
		15 min		30 min		45 min		60 min	
Compound	Dose ($\mu\text{g ml}^{-1}$)	Motility inhibition	Lysis	Motility inhibition	Lysis	Motility inhibition	Lysis	Motility inhibition	Lysis
1	5	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
	10	0±0	0±0	55±7	38±3	78±8	48±5	100±0	83±7
	25	73±7	47±6	97±2	77±5	100±0	87±8	100±0	99±1
	50	100±0	100±0	nt	nt	nt	nt	nt	nt
3	10	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
	25	0±0	0±0	15±4	0±0	35±3	0±0	45±7	0±0
	50	47±5	30±6	100±0	100±0	nt	nt	nt	nt
5	10	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
	25	41±7	0±0	90±8	51±5	93±6	69±7	100±0	88±5
	50	88±5	31±7	100±0	95±3	100±0	98±3	100±0	100±0
6	10	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
	25	0±0	0±0	0±0	0±0	19±5	0±0	52±6	0±0
	50	49±5	21±3	100±0	100±0	nt	nt	nt	nt
7	25	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
	50	30±3	0±0	59±7	33±2	78±9	49±5	81±8	51±3

^aData presented here are average value ± s.e. of at least three replications in each dose of test compound.

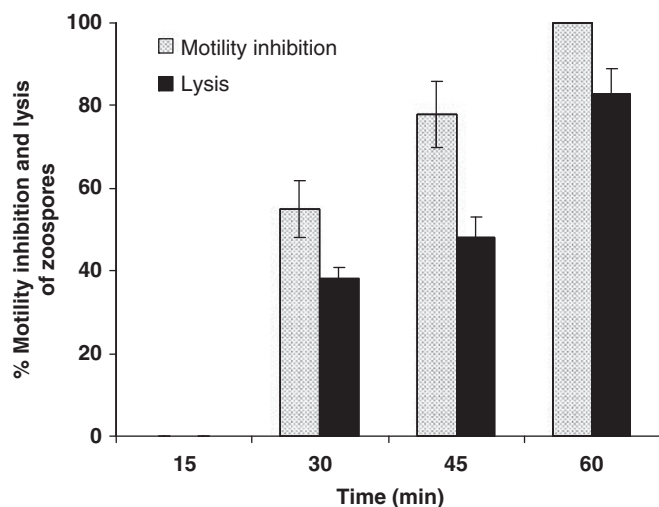


Figure 4 Motility inhibition and lysis activity of khatmiamycin (1) at $10 \mu\text{g ml}^{-1}$ (ca. $30 \mu\text{M}$) against zoospores of the grapevine downy mildew pathogen *Plasmopara viticola*.

effective control strategy against notorious peronosporomycete phytopathogens.

Khatmiamycin (1) also showed potent antibacterial activity against *Staphylococcus aureus* and *Streptomyces viridochromogenes* (Tü57) by causing growth inhibition zones 11 and 14 mm, respectively at the dose of $40 \mu\text{g}$ per paper disk. Khatmiamycin (1) belongs to the group of juglone derivatives, which are known to be cytotoxic¹⁹ or to show activity against methicillin-resistant staphylococcal strains.²⁰ In previous reports GTRI-02 (3) was found to be a lipid peroxidation inhibitor,¹³ and LL-C10037 α (6) exhibited antitumor activity. In our cytotoxicity tests using brine shrimps, 1 was, however, with 10% mortality at $10 \mu\text{g ml}^{-1}$ nearly inactive.

CONCLUSION

In this study, we isolated and characterized the new metabolite khatmiamycin (1), which showed strong motility-inhibitory and lytic effects against the zoospores of a phytopathogenic peronosporomycete *P. viticola*. This metabolite could be useful for protecting grapevine plants from the attack of downy mildew pathogen. Khatmiamycin (1) also exhibited potent antibacterial activity against *Staphylococcus aureus* and *Streptomyces viridochromogenes* (Tü 57). Further studies on the zoosporicidal mode of action of these potential zoospore-regulating compounds and their effects on other phytopathogenic peronosporomycetes are needed for considering their practical use as a naturally occurring peronosporomicidal agent.

ACKNOWLEDGEMENTS

We thank Dr H Frauendorf and R Machinek for the mass and NMR spectra, M Becker, A Kohl and F Lissy for technical assistance, the German Academic Exchange Service (DAAD) for the financial support of MAA & HYW with PhD grants, and the Alexander von Humboldt Foundation, Germany, for a fellowship to MTI. Sincere thanks are also due to Professor Beate Berkelmann, Geisenheim Research Center, Germany for kindly donating the *Plasmopara viticola* strain.

- Islam, Md. T. & Tahara, S. Chemotaxis of fungal zoospores, with special reference to *Aphanomyces cochlioides*. *Biosci. Biotechnol. Biochem.* **65**, 1933–1948 (2001).
- Dick, M. W. The Peronosporomycetes. In *The Mycota VII, Part A, Systematics and Evolution* (eds McLaughlin, D. J., McLaughlin, E. G. & Lemke, P. A.) 39–72 (Springer-Verlag, Berlin, Heidelberg, 2001).
- Kiefer, B., Riemann, M., Kassemeyer, C. B. H. & Nick, P. The host guides morphogenesis and stomatal targeting in the grapevine pathogen *Plasmopara viticola*. *Planta Med.* **215**, 387–393 (2002).
- Islam, M. T. & Tiedemann, A. V. Zoosporogenesis and differentiation of grapevine downy mildew pathogen *Plasmopara viticola* in host-free system. *Phytopathology* **98**, S72 (2008).
- Islam, M. T., Hashidoko, Y., Deora, A., Ito, T. & Tahara, S. Suppression of damping-off disease in host plants by the rhizoplane bacterium *Lysobacter* sp. strain SB-K88 is linked to plant colonization and antibiosis against soilborne Peronosporomycetes. *Appl. Environ. Microb.* **71**, 3786–3796 (2005).

- 6 Judelson, H. S. & Blanco, F. A. The spores of *Phytophthora*: weapons of plant destroyer. *Nat. Rev. Microbiol.* **3**, 47–58 (2005).
- 7 Blunt, J. W. *et al.* Marine natural products. *Nat. Prod. Rep.* **26**, 170–244 (2009).
- 8 Rahman, H. *et al.* An imidazopyridinone and further metabolites from *Streptomyces*. *Nat. Prod. Commun.* **4**, 965–970 (2009).
- 9 Islam, M. T., Ito, T., Sakasal, M. & Tahara, S. Zoosporicidal activity of polyflavonoid tannin identified in *Lannea coromandelica* stem bark against phytopathogenic oomycete *Aphanomyces cochlioides*. *J. Agric. Food Chem.* **50**, 6697–6703 (2002).
- 10 Krupa, J., Lackner, H., Jones, P. G., Schmidt-Bäse, K. & Sheldrick, G. M. Die absolute Konfiguration der Juglomycine. *Z. Naturforsch.* **44b**, 345–352 (1989).
- 11 Ozawa, M. *et al.* Structure and biosynthetic implication of (S)-NHAB, a novel shunt product, from a disruptant of the actVI-ORFA gene for actinorhodin biosynthesis in *Streptomyces coelicolor* A3(2). *Tetrahedron* **59**, 8793–8798 (2003).
- 12 Yeo, W. H. *et al.* GTRI-02, a new lipid peroxidation inhibitor from *Micromonospora* sp. SA246. *J. Antibiot.* **51**, 952–953 (1998).
- 13 Valery, M., Dembitsky, I. S. & Rozentsvet, O. A. Fatty acid amides from freshwater green alga *Rhizoclonium hieroglyphicum*. *Phytochemistry* **54**, 965–967 (2000).
- 14 Yagi, A., Makino, K. & Nishioka, I. Studies on the constituents of Aloe saponaria HAW. I. the structures of tetrahydroanthracene derivatives and the related anthraquinones. *Chem. Pharm. Bull.* **22**, 1159–1166 (1974).
- 15 Wipf, P. & Kim, Y. Synthesis of the antitumor antibiotic LL-C10037.alpha. *J. Org. Chem.* **59**, 3518–3519 (1994).
- 16 Shen, B., Whittle, Y. G., Gould, S. J. & Keszler, D. A. Structure and absolute stereochemistry of the epoxyquinol LL-C10037.alpha and related metabolites from *Streptomyces* LL-C10037. *J. Org. Chem.* **55**, 4422–4426 (1990).
- 17 Begum, P., Hashidoko, Y., Islam, M. T. & Tahara, S. Zoosporicidal activity of anacardic acids against *Aphanomyces cochlioides*. *Z. Naturforsch.* **57c**, 874–882 (2002).
- 18 Latijnhouwers, M., Ligterink, W., Vleeshouwers, V. G. A. A., van West, P. & Govers, F. A. G-alpha subunit controls zoospore motility and virulence in the potato late blight pathogen *Phytophthora infestans*. *Mol. Microbiol.* **51**, 925–936 (2004).
- 19 Celso, A. C. *et al.* Molluscicidal activity of 2-hydroxy-[1,4]naphthoquinone and derivatives. *An. Acad. Bras. Cienc.* **80**, 329–334 (2008).
- 20 Pereira, E. M. *et al.* *Tabebuia avellaneda* naphthoquinones: activity against methicillin-resistant staphylococcal strains, cytotoxic activity and *in vivo* dermal irritability analysis. *Ann. Clin. Microbiol. Antimicrob.* **5**, 5 (2006).

Supplementary Information accompanies the paper on The Journal of Antibiotics website (<http://www.nature.com/ja>)