ORIGINAL ARTICLE

Anthelmintic constituents of *Clonostachys candelabrum*

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Five diastereomeric polyketide glycosides, roselipins 3A–3E (1–5), have been isolated from the acetone extract of *Clonostachys candelabrum* on the basis of their positive anthelmintic activity. The structures of these compounds were elucidated by comparison of their NMR and MS data to those of previously reported roselipins and related structures, and were confirmed by 2D-NMR spectral analysis. Known compounds linoleic acid (6) and aurantogliocladin (7) were also isolated as active anthelmintic components, although much less potent than the roselipins.

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INTRODUCTION

Internal parasitic infection is a great threat to the productivity of the sheep and goat industry. Tremendous progress has been made in the development of anthelmintic drugs in the past 50 years. During this period, the current classes of synthetic drugs were developed, including the benzimidazoles and imidazothiazoles (such as levamisole). Another major step was achieved with the introduction of the avermectin class of macrolactones in the early 1980s. The discovery of this compound class led to anthelmintic drugs, such as ivermectin and doramectin, which have excellent broad-spectrum activity and superior potency. However, resistance to all of these classes of drugs has been observed, leading to the continuing need for further research to discover new classes of anthelmintics, especially those with novel modes of action.¹

Our strategy has been to screen extracts of bacterial and fungal fermentations as well as plants for *in vitro* activity against *Haemonchus contortus*,² which is one of the most prevalent parasitic worms that infect sheep and goats. The *in vitro* activities were then assayed *in vivo* against *Heligmosomoides polygyrus* in mice.³ This strategy led to discoveries of a number of natural products from microbial and plant origins.^{4–10} Continued efforts led to identification of an acetone extract of *Clonostachys candelabrum* that showed *in vitro* activity and was selected for further fractionation. Bioassay-guided fractionation using the *H. contortus* assay led to the isolation of active anthelmintics 1–7 (Figure 1).

RESULTS AND DISCUSSION

Compounds 1–7 were isolated from an acetone extract of a solid fermentation of *C. candelabrum* by solvent partitioning, Sephadex

LH-20 (GE Healthcare, Uppsala, Sweden) column chromatography, and finally preparative and semipreparative reversed-phase HPLC. Compounds 1–5 were separated from 6 and 7 by Sephadex LH20 chromatography. Compounds 1 and 2 were separated from 3 to 5 without much difficulty by preparative reversed-phase HPLC; however, separation of diastereomeric 1 from 2 was not possible under reversed-phase conditions. Separation of the diastereomeric mixture of 3–5 was also not possible (see experimental section for details).

Compound 6 was a clear oil that showed a peak at *m/z* 279 [M–H][–] by low-resolution MS, and end absorbance in the UV. Its ¹H NMR spectrum was compared with that of purchased linoleic acid and was found to be identical.

Compound 7 was an orange solid that showed a peak at *m/z* 197 [M+H]⁺ by low-resolution MS, gave a strong absorbance at 278 nm and a very weak absorbance at ~410 nm by UV (from HPLC-Diode Array Detector, Agilent, Santa Clara, CA, USA). The ¹H NMR showed two singlets of equal integration at δ_H 3.99 and 2.00 (CDCl₃) which, combined with the MS data, matched literature data for aurantogliocladin.^{11,12} The ¹³C NMR consisted of five signals at δ_C 184.3, 144.4, 138.9, 61.2 and 12.1, also matching literature data.¹²

The NMR, MS and UV spectra for the mixture of 1–2 were nearly identical to the mixture of 3–5. ¹H NMR features for 1–5 included alkene doublets at approximately δ_H 6.60, 5.20, 5.15 and 5.10, all with coupling constants of 8.5–10 Hz. The downfield alkene signal was due to conjugation with a carbonyl. Multiple methyl doublets were present between δ_H 0.65 and 0.90 along with four methyl singlets (three between δ_H 1.49 and 1.55 and the fourth from δ_H 1.77 to 1.78). The presence of a sugar residue was evident by the presence of an anomeric signal at approximately δ_H 4.14, and multiple other

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Figure 1 Chemical structures of compounds 1–7.

oxygenated methine signals between δ_{H} 2.85 and 4.0. The ^{13}C NMR spectrum showed presence of a carbonyl group between δ_{C} 167 and 168 for all compounds, as well as eight alkene carbons from δ_{C} 126 to 147. The anomeric carbon of the sugar residue resonated at approximately δ_{C} 96. Numerous oxygenated carbon signals were present from δ_{C} 60 to 82 in the ^{13}C NMR spectrum supporting the observed ^1H NMR assignments.

The MS data showed 1–5 to have the same formula, $\text{C}_{40}\text{H}_{70}\text{O}_{14}$, and combined with the NMR data, the class of compound was determined to be the TMC 151¹³/154¹⁴/171¹⁴ and roselipin^{15–17} antibiotics. These compounds contain a propionate polyketide chain glycosylated with a mannose, and a sugar alcohol (mannitol or arabinitol) esterified to the polyketide chain. The formulae for 1–5 along with comparison of NMR spectrum indicated that the sugar alcohol present was arabinitol. Cladionol A is another compound in this class, except that the order of hydroxyl groups and alkenes in the polyketide backbone is reversed (the double bonds are between C-4/C-5, C-8/C-9 and C-12/C-13).¹⁸ Compounds 1–5 are distinguished from cladionol A by the downfield α,β -unsaturated proton signal at δ_{H} 6.6–6.7 for H-3.

The mixture of 1 and 2 have the molecular formula of $\text{C}_{40}\text{H}_{70}\text{O}_{14}$, which was deduced from the molecular ion of 792.5088 $[\text{M}+\text{NH}_4]^+$ in the HPLC-ESIHRFTMS. Compound 1 (or 2) was identical to TMC-154, except that the latter has a methyl ether at O-4 of the mannose.¹⁴ These compounds are also identical to the TMC-171 compounds (A, B and C), except that 1 and 2 contain arabinitol instead of mannitol.¹⁴ Compounds 1 and 2 are also the 14,15-dehydro derivatives of TMC-151D and roselipin 1A/B.^{13,15–17}

Compounds 3–5 had the same molecular formula as 1 and 2, $\text{C}_{40}\text{H}_{70}\text{O}_{14}$, which was deduced from the molecular ion of 792.5095 $[\text{M}+\text{NH}_4]^+$ in the HPLC-ESIHRFTMS of the mixture. The only difference in the ^1H NMR data of 3–5 versus 1–2 was in the region of δ_{H} 4–5. The mixture of compounds 1 and 2 showed two doublets of doublets at δ_{H} 4.3 ($J=11.0$, 2.5 Hz) and 4.0 ($J=11.0$, 6.5 Hz). These protons also showed heteronuclear multiple bond coherence correlation to the carbonyl carbon, which indicated that these protons were adjacent to the ester oxygen. For compounds 3–5, these proton signals were not present, instead having new signals at δ_{H} 5.00 (ddd), 4.82 (dd, $J=7.5$, 2.0 Hz) and 4.78 (ddd), respectively, all integrating for approximately one-third of a proton. These signals indicated derivatives that were esterified at the secondary hydroxyl groups as opposed to the primary hydroxyl group as in 1 and 2. The multiplicity for the δ_{H} 4.82 signal indicated that it was the derivative esterified at O-3'.

Compounds 1–7 were evaluated for *in vitro* anthelmintic activity by the *H. contortus* assay as previously described.² Compounds 1–5 were assayed as a mixture, and reduced motility of L3 larvae with an EC_{90} (effective concentration that reduced larval motility by 90%) of $22\text{ }\mu\text{g ml}^{-1}$. Ivermectin was used as the positive control, and was more potent by approximately two orders of magnitude (EC_{90} $0.18\text{ }\mu\text{g ml}^{-1}$). Linoleic acid (6) and aurantiogliocladin (7) exhibited EC_{90} values of 930 and $420\text{ }\mu\text{g ml}^{-1}$, respectively. The mixture of 1 and 2 was assayed *in vivo* against *H. polygyrus* in mice, resulting in a 16% reduction in worm count when dosed intramuscularly at 50 mg kg^{-1} when compared with negative control. They were not tested orally. The positive control was ivermectin (81% reduction in worm count at 5 mg kg^{-1} intramuscularly).

Roselipins have been reported^{15–17} as selective inhibitors (IC₅₀ 30–50 μM) of diacylglycerol acyl transferase 2.¹⁹ Increased diacylglycerol acyl transferase 2 activity in the liver causes hepatic steatosis, whereas diacylglycerol acyl transferase 1 is known to have a role in very low-density lipoprotein. TMC-151F was reported to show cytotoxicity against several tumor cell lines.^{13,14} Roselipin 2A and 2B inhibited HIV-1 integrase with an IC₅₀ value of 8.5 μM .²⁰

In summary, we have isolated a mixture of five new roselipin derivatives, all differentiated by the position at which the polyketide chain was esterified to the arabinitol residue. Previous reported compounds in this class with the arabinitol moiety have always been esterified at the terminal oxygens; only the derivatives having a mannitol residue were esterified at the secondary hydroxyl groups. It is possible that compounds 1 and/or 2 are the naturally synthesized diastereomers, and 3–5 are artifacts formed as the result of transesterification. Linoleic acid (6) and aurantogliocladin (7) were also isolated, but were much less potent than the roselipins. This is the first report of the anthelmintic activity of the roselipin antibiotics.

METHODS

General experimental procedures

All reagents were obtained from Sigma-Aldrich (St Louis, MO, USA) and were used without further purification. IR data were obtained on a Perkin-Elmer Spectrum One (Perkin Elmer, Waltham, MA, USA). UV/Visible spectra were taken on a Perkin-Elmer Lambda 35 UV/Visible spectrometer (Perkin Elmer). NMR spectra were obtained on a Varian Inova 500 MHz spectrometer (Varian, Palo Alto, CA, USA) operating at 500 MHz for ¹H (δ 2.49) and 125 MHz for ¹³C (δ 39.5), nuclei and solvent shifts were used for internal standard. Low-resolution mass spectra were obtained on an Agilent MSD (Agilent), and high-resolution mass spectra were obtained on a Thermo Finnigan LTQ-FT (Thermo Fisher Scientific, Waltham, MA, USA) with the standard Ion Max API source (without the sweep cone) and ESI probe. Swiss Webster mice were obtained from Taconic Labs, Germantown, NY, USA. Ivermectin was used as the commercial product Ivomec Injection (1%) for Cattle and Swine (Merial, Duluth, GA, USA). Linoleic acid was obtained from Sigma-Aldrich.

Producing organism and fermentation

Clonostachys candelabrum (F-146319) was recovered from decaying leaves collected in Los Sauces, La Palma, Canary Islands, Spain. The fungus was isolated by a particle-washing technique previously described.²¹ The fungus was identified from microscopic and cultural features²² and was maintained on plates of potato dextrose agar (Difco, Lawrence, KS, USA) at 22 °C. Agar plugs were used to inoculate 250 ml baffled flasks containing 50 ml seed medium, which consisted of corn steep liquor (5 g l⁻¹), tomato paste (40 g l⁻¹), oat flour (10 g l⁻¹), glucose (10 g l⁻¹) and a trace element mix (10 ml l⁻¹). The trace element mix consisted of FeSO₄7H₂O (1 g l⁻¹), MnSO₄4H₂O (1 g l⁻¹), CuCl₂2-H₂O (25 mg l⁻¹), CaCl₂ (100 mg l⁻¹), H₃BO₃ (56 mg l⁻¹), (NH₄)₆Mo₇O₂₄4-H₂O (19 mg l⁻¹) and ZnSO₄7H₂O (200 mg l⁻¹), adjusted to pH 6.8. The flasks were incubated at 28 °C at 220 r.p.m. for 4 days.

The production phase was prepared on a solid medium in 250 ml Erlenmeyer flasks containing 14 g of millet seeds (*Panicum miliaceum*) with 30 ml of a liquid nutrient solution poured over it. The liquid nutrient solution consisted of yeast extract (16.7 g l⁻¹), sucrose (16.7 g l⁻¹), alfalfa (16.7 g l⁻¹), sodium tartrate (3.33 g l⁻¹), corn oil (3.33 ml l⁻¹) and FeSO₄7H₂O (0.33 g l⁻¹), with no pH adjustment. After inoculation, flasks were incubated without shaking at 22 °C for 28 days. Solid cultures were harvested with a spatula and stored at –20 °C before extraction.

Extraction and isolation

Fungal material (21) was initially shaken with 2 l of acetone for 3 h. The mixture was filtered and the residual solids were extracted with 1 l of 1:1 MeOH/2-butanone. The mixture was filtered and combined with the acetone extract, and evaporated under reduced pressure to ~500 ml of aqueous solution. The aqueous solution was partitioned with an equal volume of hexanes, then

with two equal portions of CHCl₃. Both hexanes and CHCl₃ extracts were separately concentrated under reduced pressure to yield 3.5 and 11.5 g of gums, respectively. The hexane extract was inactive and was not pursued. A portion (3.1 g) of the active CHCl₃ soluble material was brought up in 20 ml of MeOH and loaded onto a 21 Sephadex LH-20 column. The column was eluted with MeOH at 10 ml min⁻¹. The first 300 ml of MeOH was collected as one fraction followed by 2 min each fraction. Total elution volume of MeOH was 2.3 l. Compounds 1–5 eluted in fractions 29–38, which were combined and evaporated to give 1.00 g of white powder. Compound 6 eluted in fractions 39–54 (1.46 g of clear oil), and 7 eluted in fractions 55–61 (79 mg of orange solid). The combined fraction 29–38 was brought up in 8 ml of MeOH and injected in eight 1-ml injections onto preparative HPLC (Waters, Milford, MA, USA) XTerra RP C₁₈, 300 × 19 mm, 7 μm , 10 ml min⁻¹, 25–90% MeCN in H₂O, both containing 0.1% TFA, gradient time 40 min and UV detection at 210 nm. Fractions 60–66 were lyophilized to afford 139.6 mg of compounds 3–5, and fractions 67–74 were similarly lyophilized to give 454.8 mg of compounds 1–2 both as powder.

Roselipins 3A and 3B (1, 2)

White solid: IR (ZnSe film) ν_{max} 3348, 2958, 2922, 2871, 1671, 1642, 1453, 1373, 1312, 1225, 1063, 1025, 944, 868, 798, 750 cm⁻¹; UV (MeOH) λ_{max} (log ϵ) 202 (4.43), 220 (sh, ~4.12) nm; ¹H-NMR (DMSO-*d*₆, 500 MHz) δ 6.66 and 6.64 (1H, dq, *J*=10.0, 1.5 Hz, H-3), 5.23 (1H, d, *J*=8.5 Hz, H-11), 5.18 (1H, d, *J*=9.0 Hz, H-7), 5.11 (1H, d, *J*=9.5 Hz, H-15), 4.30 (1H, dd, *J*=11.0, 2.5 Hz, H-1'a), 4.14 (1H, d, *J*=<1, H-1''), 3.99 (1H, dd, *J*=11.0, 6.5, H-1'b), 3.89 (1H, d, *J*=8.5, H-13), 3.72 (1H, ddd, *J*=9.0, 7.0, 2.5, H-2'), 3.66 (2H, m, H-5, H-6''b), 3.59 (1H, dd, *J*=11.0, 3.0, H-5'a), 3.52 (2H, m, H-9, H-2''), 3.35–3.47 (4H, m, H-6'a, H-3', H-4', H-5'b), 3.25 (1H, t, *J*=9.5, H-4''), 3.15 (1H, dd, *J*=9.5, 3.0, H-3''), 2.85 (1H, ddd, *J*=9.5, 6.5, 2.5, H-5''), 2.56 (2H, m, H-4 and H-12), 2.50 (overlapping with solvent, H-16), 2.42 (1H, m, H-8), 1.78 and 1.77 (3H, d, *J*=1.5 Hz, H-21), 1.55 (3H, s, H-23), 1.53 (3H, s, H-25), 1.50 (3H, s, H-27), 0.90 (3H, d, *J*=7.0 Hz, H-28), 0.80, (3H, t, *J*=7.5 Hz, H-20), 0.79 (3H, d, *J*=7.0 Hz, H-29), 0.75 (3H, d, *J*=6.5 Hz, H-26), 0.68 (3H, d, *J*=7.0 Hz, H-24); ¹³C-NMR (DMSO-*d*₆, 125 MHz) δ 167.7 and 167.4 (C-1), 146.7 and 146.5 (C-3), 137.7 (C-15), 136.1 and 136.0 (C-6), 135.6 (C-10), 131.4 (C-7), 130.7 (C-11), 129.9 (C-14), 126.6 and 126.5 (C-2), 96.2 (C-1''), 85.7 (C-13), 81.2 (C-9), 80.9 and 80.8 (C-5), 77.8 (C-5''), 74.2 (C-3''), 71.0 × 2, 70.8, 70.4, 68.6, 67.4 (C-4), 67.3, 67.1 (C-1'), 66.1, 63.6, 62.8, 61.3 (C-6''), 44.1 (C-17), 36.9 (C-4), 36.1 (C-8), 33.8 (C-12), 32.1 (C-18), 29.9 (C-19), 29.4 (C-16), 21.8 (C-28), 18.8 (C-29), 17.5 (C-24), 17.2 (C-26), 16.5 (C-22), 12.6 (C-21), 11.4, 11.2 (×2), 11.1 (C-20, C-23, C-25, C-27); HRESIFTMS *m/z* 792.5088 [M+NH₄]⁺ (calcd for C₄₀H₇₄NO₁₄, 792.5109 [M+NH₄]⁺).

Roselipins 3C, 3D and 3E (3–5)

White solid: ¹H-NMR (DMSO-*d*₆, 500 MHz) δ 6.67 (1H, d, *J*=9.5 Hz, H-3), 5.23 (1H, d, *J*=8.5 Hz, H-11), 5.17 (1H, d, *J*=8.5 Hz, H-7), 5.11 (1H, d, *J*=9.5 Hz, H-15), 5.00 (H, ddd, *J*=7.5, 5.5, 2.0 Hz, H-2' or H-4' (for H-2' or H-4' ester)), 4.82 (H, dd, *J*=7.5, 2.0 Hz, H-3' for H-3' ester), 4.78 (H, ddd, *J*=7.5, 4.5, 3.0 Hz, H-2' or H-4' (for H-2' or H-4' ester)), 4.14 (1H, d, *J*< 2 Hz, H-1''), 3.88 (1H, d, *J*=8.5, H-13), 3.65 (2H, m, H-5, H-6''b), 3.52 (2H, m, H-9, H-2''), 3.35–3.47 (m, H-6'a, arabinitol protons), 3.24 (1H, t, *J*=9.5, H-4''), 3.14 (1H, dd, *J*=9.5, 3.0, H-3''), 2.84 (1H, ddd, *J*=9.0, 6.5, 2.0, H-5''), 2.55 (2H, m, H-4 and H-12), 2.50 (overlapping with solvent, H-16), 2.42 (1H, m, H-8), 1.76 (3H, d, *J*=1.5 Hz, H-21), 1.54 (3H, s, H-23), 1.53 (3H, s, H-25), 1.49 (3H, s, H-27), 0.90 (3H, d, *J*=6.5 Hz, H-28), 0.80, (3H, t, *J*=7.5 Hz, H-20), 0.78 (3H, d, *J*=7.5 Hz, H-29), 0.75 (3H, d, *J*=6.5 Hz, H-26), 0.68 (3H, d, *J*=6.5 Hz, H-24); HRESIFTMS (as mixture of 3–5) *m/z* 792.5095 [M+NH₄]⁺ (calcd for C₄₀H₇₄NO₁₄, 792.5109 [M+NH₄]⁺).

Biological assay

The *in vitro* assay against *H. contortus* was performed as described by Michael *et al.*² The *in vivo* mouse assay was modified from Fonseca-Salamanca *et al.*³ and performed as follows: Swiss Webster mice (~30 g) were inoculated with 200–400 L3 *H. polygyrus* larvae. The mice were checked for infection around day 12 after inoculation, then dosed intramuscularly with test compound in triplicate. On day 3 after treatment, mice were killed and the intestine was

collected (from below stomach to above cecum), opened, and placed in 5 ml of sterile water. Mucosa was scraped and rinsed through a 200-mesh screen. The rinsate was then examined for presence of worms, and worms were counted. Worm counts for treated mice were then compared with infected, untreated mice as negative controls and infected mice treated with ivermectin (5 mg kg⁻¹) as positive controls.

- 1 McKellar, Q. A. & Jackson, F. Veterinary anthelmintics: old and new. *Trends. Parasitol.* **20**, 456–461 (2004).
- 2 Michael, B., Meinke, P. T. & Shoop, W. Comparison of ivermectin, doramectin, selamectin, and eleven intermediates in a nematode larval development assay. *J. Parasitol.* **87**, 692–696 (2001).
- 3 Fonseca-Salamanca, F., Martinez-Grueiro, M. M. & Martinez-Fernandez, A. R. Nematocidal activity of nitazoxanide in laboratory models. *Parasitol. Res.* **91**, 321–324 (2003).
- 4 Ayers, S., Zink, D. L., Brand, R., Pretorius, S., Stevenson, D. & Singh, S. B. Struthiolanone: a flavanone-resveratrol adduct from *Struthiola argentea*. *Nat. Prod. Commun.* **3**, 189–192 (2008).
- 5 Ayers, S. *et al.* Anthelmintic macrolactams from *Nonomuraea turkmeniaca* MA7364. *J. Nat. Prod.* **70**, 1371–1373 (2007).
- 6 Ayers, S. *et al.* Scutiaquinones a and b, perylenequinones from the roots of *Scutia myrtina* with anthelmintic activity. *J. Nat. Prod.* **70**, 425–427 (2007).
- 7 Ayers, S. *et al.* Anthelmintic activity of aporphine alkaloids from *Cissampelos capensis*. *Planta Med.* **73**, 296–297 (2007).
- 8 Ayers, S. *et al.* Anthelmintic macrolactams from *Nonomuraea turkmeniaca* MA7381. *J. Antibiot.* **61**, 59–62 (2008).
- 9 Ayers, S. *et al.* Noreupenifeldin, a tropolone from an unidentified ascomycete. *J. Nat. Prod.* **71**, 457–459 (2008).
- 10 Ayers, S. *et al.* Flavones from *Struthiola argentea* with anthelmintic activity *in vitro*. *Phytochem.* **69**, 541–545 (2008).
- 11 Savoie, J. Y. & Brassard, P. Preparation de quelques cyclohexène-2 diones-1,4 et de leurs dérivés. *Can. J. Chem.* **49**, 3515–3523 (1971).
- 12 Roth, L. & Rupp, G. *Roth Collection of Natural Products Data*. ed. 1–142. (VCH: Weinheim, 1995.) Vol. II.
- 13 Kohno, J. *et al.* Isolation and structure determination of TMC-151s: novel polyketide antibiotics from *Gliocladium catenulatum* Gilman & Abbott tc 1280. *Tetrahedron* **55**, 7771–7786 (1999).
- 14 Kohno, J. *et al.* TMC-171A,B,C and TMC-154, novel polyketide antibiotics produced by *Gliocladium* sp. TC 1304 and TC 1282. *J. Antibiot.* **52**, 1114–1123 (1999).
- 15 Omura, S., Tomoda, H., Tabata, N., Ohyama, Y., Abe, T. & Namikoshi, M. Roselipins, novel fungal metabolites having a highly methylated fatty acid modified with a mannose and an arabinitol. *J. Antibiot.* **52**, 586–589 (1999).
- 16 Tabata, N., Ohyama, Y., Tomoda, H., Abe, T., Namikoshi, M. & Omura, S. Structure elucidation of roselipins, inhibitors of diacylglycerol acyltransferase produced by *Gliocladium roseum* KF-1040. *J. Antibiot.* **52**, 815–826 (1999).
- 17 Tomoda, H. *et al.* Roselipins, inhibitors of diacylglycerol acyltransferase, produced by *Gliocladium roseum* KF-1040. *J. Antibiot.* **52**, 689–694 (1999).
- 18 Kasai, Y., Komatsu, K., Shigemori, H., Tsuda, M., Mikami, Y. & Kobayashi, J. Cladionol a, a polyketide glycoside from marine-derived fungus *Gliocladium* species. *J. Nat. Prod.* **68**, 777–779 (2005).
- 19 Inokoshi, J., Kawamoto, K., Takagi, Y., Matsuhama, M., Omura, S. & Tomoda, H. Expression of two human Acyl-CoA: diacylglycerol acyltransferase isozymes in yeast and selectivity of microbial inhibitors toward the isozymes. *J. Antibiot.* **62**, 51–54 (2009).
- 20 Singh, S. B. *et al.* Isolation, structure, and HIV-1-integrase inhibitory activity of structurally diverse fungal metabolites. *J. Ind. Microbiol. Biotechnol.* **30**, 721–731 (2003).
- 21 Bills, G. F. & Polishook, J. D. Abundance and diversity of microfungi in leaf-litter of a lowland rain-forest in costa-rica. *Mycologia* **86**, 187–198 (1994).
- 22 Schroers, H. J. A monograph of bionectria ascomycota, hypocreales, bionectriaceae and its clonostachys anamorphs. *Studies Mycol.* **46**, 1–214 (2001).