Supplementary Information

Structure and Absolute Configuration of Secondary Metabolites from Two Strains of *Streptomyces chartreusis* Associated with Attine Ants

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Figure S1. Antagonist bioassay of *Streptomyces chartreusis* AC70 against *Escovopsis* sp. (A) Bacterium *versus* *Escovopsis* sp., (B) growth control of *Escovopsis* sp., (C) positive control miconazole (50 µg) against *Escovopsis* sp. Assays were carried out in ISP-2 agar plates (250 × 20 mm) and 150 µL of a spore solution (1 × 10⁶) of the pathogenic fungus. One plug of bacterium was placed in the center of the plate. The Petri dishes were incubated during 7 days at 30 °C.

Figure S2. Antagonist bioassay of *Streptomyces chartreusis* ICBG323 against *Escovopsis* sp. using soft-agar overlay technique. For this, a previously prepared spore suspension aliquot was added in a concentration of about 10⁶ spores per mL to soft-agar and this was poured onto Petri dishes containing a thin layer of ISP2 agar medium. After complete softening of the soft-agar, one plug of bacterium ICBG323 was placed in the center of plate. The plates were incubated at 28 °C and the readings were performed on day 7, in order to verify the appearance of inhibition halos.
Figure S3. Chromatogram of a fraction containing streptazolin (1), its E-isomer (2), strepchazolin A (3) and strepchazolin B (4). Column used: Phenomenex C6-phenyl column (5 µm, 250 × 4.6 mm). High-performance liquid chromatography (HPLC) method: isocratic flow of 5% CH3CN at 6 mL min⁻¹.

Figure S4. High-resolution electrospray ionization mass spectrometry (HRESIMS) of streptazolin (1).

Figure S5. ¹H nuclear magnetic resonance (NMR) of streptazolin (1) (500 MHz, CDC13).
Figure S6. HRESIMS of E-isomer of streptazolin (2).

Figure S7. $^1$H NMR of E-isomer of streptazolin (2) (500 MHz, CDCl$_3$).
Figure S8. HRESIMS of compound 3.

Figure S9. $^1$H NMR of compound 3 (500 MHz, CDCl$_3$).
Figure S10. Gradient heteronuclear single quantum coherence (gHSQC) of compound 3 (500 MHz, CDCl$_3$).

Figure S11. Gradient-selected heteronuclear multiple bond coherence (gHMBC) of compound 3 (500 MHz, CDCl$_3$).
Figure S12. Gradient-selected correlation spectroscopy (gCOSY) of compound 3 (500 MHz, CDCl₃).

Figure S13. Nuclear Overhauser spectroscopy (NOESY) 1D spectrum of compound 3 (500 MHz, CDCl₃).
Figure S14. HRESIMS of compound 4.

Figure S15. $^1$H NMR spectrum of compound 4 (500 MHz, CDCl$_3$).
Figure S16. Ultraviolet (UV) spectrum of compound 4 in MeOH.

Figure S17. gHSQC of compound 4 (500 MHz, CDCl₃).
Figure S18. gHMBC of compound 4 (500 MHz, CDCl$_3$).

Figure S19. gHMBC of compound 4 (500 MHz, CDCl$_3$).
Figure S20. gCOSY of compound 4 (500 MHz, CDCl₃).

Figure S21. ¹³C NMR of compound 4 (100 MHz, CDCl₃).
Figure S22. Distortionless enhancement by polarization transfer (DEPT)-135 of compound 4 (100 MHz, CDCl₃).

Figure S23. NOESY 1D spectra of compound 4 (500 MHz, CDCl₃).
Figure S24. Gas chromatography mass spectrometry (GC-MS) spectra of fraction B1.2.

Figure S25. GC-MS spectra of active fraction of crude extract of *Streptomyces chartreusis* ICBG323 against *Escovopsis* sp.
Figure S26. (A) Antagonist activity of fraction B1.2 (100 µg) against *Escovopsis* sp., (B) Miconazole (50 µg) *versus* *Escovopsis* sp., (C) negative control. Samples were placed in a paper disk in the edge of ISP-2 agar plate and fungal strain was then point-inoculated in the center of plate. The Petri dishes were incubated during 7 days at 30 °C.

Figure S27. (A) Antagonism activity of active fraction, containing 5 as major compound, (70.5 µg) of crude extract of *Streptomyces chartreusis* ICBG323 against *Escovopsis* sp. using soft agar overlay technique.1,2 (B) Miconazole (50 µg) *versus* *Escovopsis* sp., (C) negative control. For this, a previously prepared spore suspension aliquot was added in a concentration of about 10⁶ spores mL⁻¹ to soft-agar and this was poured onto Petri dishes containing a thin layer of ISP2 agar medium. After complete softening of the soft agar, the compound or fraction was placed directly in the center of plate. The plates were incubated at 28 °C and the readings were performed on day 7, in order to verify the appearance of inhibition halos.

References