Supporting Information

Peptaibols, Tetramic Acid Derivatives, Isocoumarins and Sesquiterpenes from a Bionectria sp. (MSX 47401)

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- Producing Organism and Fermentation.

- Marfey’s Analysis of 1 and 3.

- Table S1. HRMS Data and [α]D Values for Compounds 4–6

- Figure S1. Phylogram of the most likely tree (-lnL = 2689.70) from a RAxML analysis of 60 taxa based on complete Internal Transcribe Spacer regions 1 & 2 including 5.8 S rRNA gene (470 bp). Numbers above the nodes refer to RAxML bootstrap support values ≥ 70% based on 1000 replicates. MSX 47401 is shown in bold and shows phylogenetic affinities to two strains of Bionectria rossmaniae (AF358227 and AF210665). However, more strains and/or morphology may need to be examined to determine if MSX 47401 is conspecific with B. rossmaniae.

- Figure S2. 1H (500 MHz) and 13C (125 MHz) NMR of AGI-7 (6) in DMSO-d6.

- Figure S3. 1H (500 MHz) NMR spectra of virgineone (4) in methanol-d4 (top) and DMSO-d6 (bottom).

- Figure S4. 13C (125 MHz) NMR spectra of virgineone (4) in methanol-d4 (top) and DMSO-d6 (bottom).

- Figure S5. 1H (500 MHz) and 13C (125 MHz) NMR spectra of virgineone aglycone (5) in DMSO-d6.
- **Figure S6.** $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR spectra of 5,6-dihydroxybisabolol (7) in methanol-$d_4$.

- **Figure S7.** $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR spectra of 1,2-dehydrovirgineone (2) in methanol-$d_4$.

- **Figure S8.** HSQC (top; (500 MHz) and HMBC (bottom; (500 MHz) NMR spectra of 1,2-dehydrovirgineone (2) in methanol-$d_4$.

- **Figure S9.** COSY (500 MHz) NMR spectrum of 1-2-dehydrovirgineone (2) in methanol-$d_4$.

- **Figure S10.** $^1$H (950 MHz) and $^{13}$C (150 MHz) NMR spectra of clonostachin B (1) in methanol-$d_3$.

- **Figure S11.** NOESY (top; 950 MHz) and TOCSY (bottom; 950 MHz) NMR spectra of clonostachin B (1) in methanol-$d_3$.

- **Figure S12.** HMBC (700 MHz) NMR spectrum of clonostachin B (1) in methanol-$d_3$.

- **Figure S13.** $^1$H (950 MHz) and $^{13}$C (150 MHz) NMR spectra of clonostachin (3) in methanol-$d_3$.

- **Figure S14.** NOESY (top; 950 MHz and TOCSY (bottom; 950 MHz) NMR spectra of clonostachin (3) in methanol-$d_3$.

- **Figure S15.** Marfey’s analysis of clonostachin (3) and clonostachin B (1), indicating the exchanged residues of Aib11 in 3 for Iva11 in 1.
Producing Organism and Fermentation. The fungal strain MSX 47401 culture was stored on a malt extract slant and was transferred periodically. A fresh culture was grown on a similar slant, and a piece was transferred to a medium containing 2% soy peptone, 2% dextrose, and 1% yeast extract (YESD media). Following incubation (7 d) at 22 °C with agitation, the culture was used to inoculate 50 mL of a rice medium, prepared using rice to which was added a vitamin solution and twice the volume of rice with H₂O in a 250 mL Erlenmeyer flask. This was incubated at 22 °C until the culture showed good growth (approximately 14 d). The scale-up culture was grown in a 2.8 L Fernbach flask containing 150 g of rice and 300 mL of H₂O and was inoculated using a seed culture grown in YESD medium. This was incubated at 22 °C for 14 d. For extraction of genomic DNA of fungal strain MSX 47401, mycelium from axenic cultures grown in YESD broth was scraped with a sterile scalpel and ground to a fine powder in liquid N₂ using a mortar and pestle. Approximately 400 µL of AP1 buffer from the DNAeasy Plant Mini Kit (QIAGEN Inc., Valencia, CA) was added to the mycelia powder, and DNA was extracted following the manufacturer’s instructions. The DNA was eluted in approximately 25–30 µl distilled H₂O. The complete ITS region, along with the partial region of divergent domains D1/D2 of the large subunit of the 28S nuclear ribosomal DNA ( LSU), were amplified with ITS1F and LR3 by PCR using puReTaq™ Ready-To-Go PCR beads (Amersham Biosciences Corp., Piscataway, NY). The PCR products were sequenced subsequently in a 11 µL sequencing reaction with BigDye® Terminators v3.1 (Applied Biosystems, Foster City, CA) using ITS primers ITS1F and ITS4 and LSU primers LROR and LR3. For PCR, the following protocol was used: initial denaturation at 95 °C for 5 min, followed by 35 or 40 cycles of 95 °C for 30 s, 41 or 50 °C for 15 s, and 72 °C for 1 min with a final extension step of 72 °C for 10 min. To enhance the PCR reactions, 2.5 µL of BSA (bovine serum albumin, New England Biolabs, Ipswich, MA) and/or 2.5 µL of DMSO (dimethyl sulfoxide, Fisher Scientific, Pittsburgh, PA) were added. The PCR products were purified to remove excess primers, dNTPs, and nonspecific amplification products with the QIAquick PCR Purification Kit (QIAGEN Inc.). Sequences were generated on an Applied Biosystems 3730XL high-throughput capillary sequencer at the University of Illinois Urbana-Champaign Biotech facility.

Marfey’s Analysis of 1 and 3. Approximately 0.2 mg of each amino acid standard was weighed into separate glass 2-mL reaction vials. To each standard was added 50 µL of H₂O, 20 µL of 1 M NaHCO₃, and 100 µL 1% Marfey’s reagent in acetone. The reaction mixtures were agitated at 40 °C for 1 h. The reactions were halted by the addition of 10 µL of 2 N HCl. The product of the reactions was dried under a stream of air and dissolved in ~1.7 mL of MeOH. Each derivatized standard was injected individually (0.7 µL) onto the UPLC. Also, aliquots of all of the derivatized standards were combined to give a mixed standard, which was injected just prior to the digested and derivatized peptaibols. UPLC conditions were 15–80% CH₃CN in H₂O over 10 min on the aforementioned BEH column and eluent monitored at 340 nm. To generate the digested and derivatized peptaibols, approximately 0.25 mg of compounds 1 and 3 were weighed separately into 2-mL reaction vials, to which was added 0.5 mL of 6N HCl. The compounds were hydrolyzed at 110 °C for 24 h, at which time they were evaporated under a stream of air. To each hydrolysis product was then added 25 µL H₂O, 10 µL 1 M NaHCO₃, and 50 µL of 1% Marfey’s reagent in acetone. The reaction mixtures were agitated at 40 °C for 1 h. The reactions were halted by the addition of 5µL of 2 N HCl. The mixtures were dried under a stream of air and brought up in ~200 µl of MeOH and injected onto the UPLC with the use of the same conditions as for the standards.
<table>
<thead>
<tr>
<th>Compound</th>
<th>HRESIMS (m/z)</th>
<th>[α]_D (in MeOH)</th>
</tr>
</thead>
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<tr>
<td>Virgineone (4)</td>
<td>750.4415</td>
<td>750.4423</td>
</tr>
<tr>
<td>Virgineone aglycone (5)</td>
<td>588.3878</td>
<td>588.3895</td>
</tr>
<tr>
<td>AGI-7 (6)</td>
<td>221.0444</td>
<td>221.0445</td>
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</tbody>
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![NMR Spectra Image](image-url)
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Sample 01008-91-5
NOESY
August 4, 2011

Sample 01008-91-5
TOCSY
August 4, 2011
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