Brocaenols A-C: Novel Polyketides from a Marine-derived *Penicillium brocae*

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Figure S1. ^1H NMR spectrum of Brocaenol A (**1**)

Figure S2. ^{13}C NMR spectrum of Brocaenol A (1)

Figure S3. ^1H NMR spectrum of Brocaenol B (2)

Figure S4. ^{13}C NMR spectrum of Brocaenol B (2)

Figure S5. ^1H NMR spectrum of Brocaenol C (3)

Figure S6. ^{13}C NMR spectrum of Brocaenol C (3) in CD_3OD

Figure S7. ^{13}C NMR spectrum of Brocaenol C (3) in DMSO-d_6

Taxonomic Characterization

Taxonomic characterization of the fungus was based on both morphology and sequence analysis of the ITS regions. We first tried to identify F97S76 through molecular taxonomy. The genomic DNA was isolated and the ITS1-5.8S-ITS2 region (ITS region) was PCR-amplified using the primers ITS1 and ITS4.¹ After editing out the partial 18S and 28S rDNA, the ITS region of F97S76 was 503 base pairs in length. A nucleotide Blast search (NCBI 2.2) was performed to compare this sequence to other sequences in the GenBank database. The most closely related species was *Penicillium adametzii* NRRL 737 (GenBank accession AF033401) with a homology of 93.2% (465/499 base pairs).

Since the homology was relatively low, isolate F97S76 was examined morphologically in an attempt to further characterize the species. Isolate F97S76 was inoculated onto Czapek's agar medium, Czapek yeast-extract agar, and malt extract agar. The plates were incubated at 22 °C for seven days and examined microscopically. Isolate F97S76 was clearly monoverticillate, but did not match any monoverticillate descriptions found in the *Manual and atlas of the Penicillia*.² At this point, it appeared that the species was undescribed. However, comparison with the personal collection of Stephen Peterson (National Center for Agricultural Utilization Research, US Department of Agriculture) revealed that the ITS region of F97S76 was identical to a newly described species *Penicillium brocae* (GenBank accession AF484396).³ Therefore, we have decided that F97S76 is a *Penicillium brocae* isolate.

References

- (1) White, T. J.; Bruns, T.; Lee, S.; Taylor, J. *PCR Protoc.: Guide Methods Appl.* **1990**, 315-322.
- (2) Ramirez, C. *Manual and Atlas of the Penicillia*; Elsevier Biomedical Press: New York, 1982.
- (3) Peterson, S. W.; Perez, J.; Vega, F. E.; Infante, F. *Mycologia* **2002**, *in press*.

Preparation of the *S*-MTPA ester of 1: 2.2 mg of **1** was dissolved in 100 μ L of anhydrous pyridine followed by the addition of 13 μ L of *R*-methoxy(trifluoromethyl)phenylacetyl chloride (MTPA-Cl). The reaction was allowed to stand at 20°C for 1 hr under an atmosphere of argon. The reaction mixture was dried under a stream of N₂, and subsequently, the *S*-MTPA ester was partially purified by HPLC using isopropanol and hexanes (2% IPA to 50% IPA, 25 min. linear gradient) on SiO₂. Final purification was performed by HPLC using isopropanol and hexanes (2% IPA to 10% IPA, 15 min. linear gradient, flow 4.0 mL/min) on SiO₂ (10 mm $\times$ 250 mm). ¹H NMR (CDCl₃) δ 7.75 (2H, m, mtpa-Ar-H), 7.65 (1H, t, *J* = 8.5 Hz, H-3), 7.53 (2H, m, mtpa-Ar-H), 7.46-7.41 (4H, m, mtpa-Ar-H), 7.36 (1H, d, *J* = 8.5 Hz, H-2), 7.34 (2H, m, mtpa-Ar-H), 6.96 (1H, d, *J* = 8.5 Hz, H-4), 6.54 (1H, bd, *J* = 1.0 Hz, H-7), 5.41 (1H, bs, H-5), 3.65 (3H, s, H-13), 3.52 (3H, s, mtpa-Me), 3.50 (3H, s, mtpa-Me), 1.92 (3H, bs, H-11); CIMS [M+H]⁺ *m/z* 767.2.

Preparation of the *R*-MTPA ester of 1: Similar conditions did not effect esterification with *S*-MTPA-Cl and a different procedure was used. 3.2 mg was dissolved in 50 μ L of anhydrous pyridine followed by the addition of 20 μ L of *S*-MTPA-Cl and the reaction was allowed to stand for 3.5 hrs at 20°C under an atmosphere of argon. The reaction was dried under a stream of N₂, and subsequently, the *R*-MTPA ester was partially purified by running the sample over a 3 cm plug of SiO₂ using 30% IPA in Hexanes. Final purification was performed by HPLC using isopropanol and hexanes (2% IPA to 10% IPA, 15 min. linear gradient, flow 4.0 mL/min) on SiO₂ (10 mm $\times$ 250 mm). ¹H NMR (CDCl₃) δ 7.72 (2H, m, mtpa-Ar-H), 7.62 (1H, t, *J* = 8.5 Hz, H-3), 7.51 (2H, bs, mtpa-Ar-H), 7.43 (3H, bm, mtpa-Ar-H and H-2), 7.35 (4H, m, mtpa-Ar-H), 6.87 (1H, d, *J* = 8.5 Hz, H-4), 6.49 (1H, bs, H-7), 4.58 (1H, bs, H-5), 3.81 (3H, s, H-13), 3.52 (3H, s, mtpa-Me), 3.50 (3H, s, mtpa-Me), 1.76 (3H, bs, H-11); CIMS [M+H]⁺ *m/z* 767.2.

Acetylation of 1: 4.0 mg of **1** was dissolved in 100 μ L of pyridine followed by a 50 μ L addition of acetic anhydride. The solution was stirred at room temperature for 24 hours and subsequently dried under vacuum. The sample was purified by flash silica gel chromatography to yield 3.7 mg of the diacetate. UV (MeOH) λ_{max} (log ϵ) 212 (4.09), 226 (s) (4.04), 270 (3.57), 292 (3.52); ¹H NMR (CDCl₃) δ 7.62 (1H, t, *J* = 7.6 Hz, H-3), 7.31 (1H, dd, *J* = 7.6, 1.2 Hz, H-2), 6.99 (1H, dd, *J* = 7.6, 1.2 Hz, H-4), 6.52 (1H, bd, *J* = 1.6 Hz, H-7), 5.39 (1H, bs, H-5), 4.47 (1H, bs, OH-8), 3.69 (3H, s, H-13), 2.34 (3H, s), 2.04 (3H, s), 1.83 (3H, bd, *J* = 1.6 Hz, H-11); ¹³C NMR (CDCl₃) δ 178.4, 173.0, 169.7, 169.5, 161.9, 154.1, 149.2, 138.8, 133.7, 126.3, 119.6, 115.4, 114.8, 104.4, 74.6, 73.1, 53.4, 21.1, 20.6, 16.9; HREIMS *m/z* 418.0907 (M⁺ calcd for C₂₀H₁₈O₁₀, 418.0900).