Isolation and Structure Elucidation by LC-MS-SPE/NMR: PR Toxin- and Cuspidatol-Related Eremophilane Sesquiterpenes from *Penicillium roqueforti*

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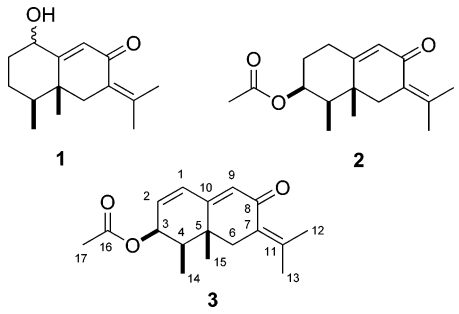
Three eremophilane sesquiterpenes (**1**, **2**, and **3**) were isolated from *Penicillium roqueforti* DAOM 232127, and their structures were established. The new (3*S*)-3-acetoxyeremophil-1(2),7(11),9(10)-trien-8-one (**3**) is a likely biosynthetic precursor of PR toxin. 1-Hydroxyeremophil-7(11),9(10)-dien-8-one (**1**) is related to the immunosuppressant cuspidatol. The application of semihyphenated LC-MS-SPE/NMR to rapidly identify, purify, and elucidate the structures of **1**, **2**, and **3** is described.

Natural products have a proven track record as a source of therapeutic compounds and lead structures for medicinal chemistry.[1] However, the modern drug discovery process[2] and the trend toward high-throughput screening (HTS) and structural scaffolds amenable to rapid synthesis of analogues[3] have constrained the role of natural products.[4,5] Novel approaches are clearly needed for natural products to successfully run the gauntlet of industrial drug discovery.[6,7] Timely dereplication, isolation, and structural elucidation of compounds are initial prerequisites, which can be accomplished by the emerging hyphenated technique of LC-MS-SPE-NMR (liquid chromatography−mass spectrometry−solid-phase extraction−nuclear magnetic resonance).[8]

Microfungi are a particularly interesting source of secondary metabolites, which have historically led to the important discoveries of β-lactams, echinocandins, statins, and cyclosporins. Many microfungi can be cultivated and are prolific producers of mycotoxins and other potent compounds of importance to our health and economy. Existing scientific knowledge and novel technology present the opportunity to conduct chemotaxonomy-driven studies of these biological small-molecule libraries.[9]

Penicillium roqueforti DAOM 232127 was obtained from contaminated silage associated with ill thrift and reduced milk production of cows in Québec. This particular strain was also incorporated into a larger chemotaxonomic study that investigated the metabolites of many silage-derived strains from areas in Scandinavia and eastern Canada, where silage toxicosis is regularly observed.[10,11] Since initial HPLC results showed the presence of relatively large amounts of unknowns, it was chosen for further studies. Semihyphenated LC-MS-SPE/NMR was used to identify, isolate, and elucidate the structures of three eremophilane sesquiterpenes, **1**−**3**. This is the first report of these compounds from the *Penicillium* species, but while **3** is a new structure, **1** and **2** were previously implicated in phytochemical and synthetic studies.[12,13]

For the purpose of chemotaxonomy and dereplication of known compounds, the initial analysis of the fungal extract was conducted by a standardized LC-MS method.[11,14] This approach served to identify many metabolites, such as roquefortines A, C, and D, citreoisocoumarin, PR toxin, eremofortin C, and mycophenolic acid, but the complexity of the sample and isobaric chromatographic peaks made it difficult to discriminate between possible structures of unknown compounds. In an attempt to gather NMR data on some unidentified metabolite peaks, a method was developed to perform stop-flow LC-NMR. However, with deuterated solvents, it proved difficult to modify the chromatography to reflect the quality and elution order observed in analytical LC-MS. Lack of real-time MS data complicated the proper selection of peaks for stop-flow NMR analysis, and the inherent time constraints led to acquisition of only ^{1}H NMR and gCOSY spectra. While sufficient to identify a few known compounds, the method was inadequate for structural elucidation of unknowns. The requirements of minimal sample preparation, robust chromatography, real-time MS data, and long acquisition times of multiple 2D NMR spectra led us to implement LC-MS-SPE/NMR as a tool for isolation and structural elucidation. The option of flow-cell NMR was abandoned in favor of elution into 3 mm NMR tubes, which allowed us to conduct simultaneous and prolonged experiments with existing NMR instrumentation. As a proof of concept, 5 μg of roquefortine C from a single injection of the fungal extract was isolated by MS-guided SPE-trapping and afforded excellent NMR spectra for structural confirmation (data not shown).



Due to apparent similarities, the peaks of the unknown compounds **1**, **2**, and **3** were targeted for isolation: **1** and **2** had very similar UV spectra and **2** and **3** differed by only 2 mass units. Initial NMR analysis of the three compounds in CD_3CN confirmed their structural similarity, but the residual solvent and water peaks overlapped with important resonances. A small aliquot for high-resolution ESIMS (HRESIMS) and a solvent switch to C_6D_6 were required for complete structural elucidation. While gHSQC and gHMBC spectra were acquired for **1** and **3**, poor signal-to-noise levels and the need for increased sensitivity led to the adaptation of, in lieu of sensitivity-enhanced gradient experiments, non-gradient-selected HSQC and HMBC experiments for **2**.

In conjunction with integration of ^{1}H NMR signals, multiplicities, and approximation of the number of carbon atoms from (g)HSQC and (g)HMBC spectra, HRESIMS established the formulas of **1**−**3**. One degree of unsaturation separated **2** and **3**, while a difference of 42 mass units suggested that **1** was a nonacetylated version of **2**. Comparison of the ^{1}H NMR spectra of **2** and **3** confirmed the presence of an additional double bond in **3** and recognition of the

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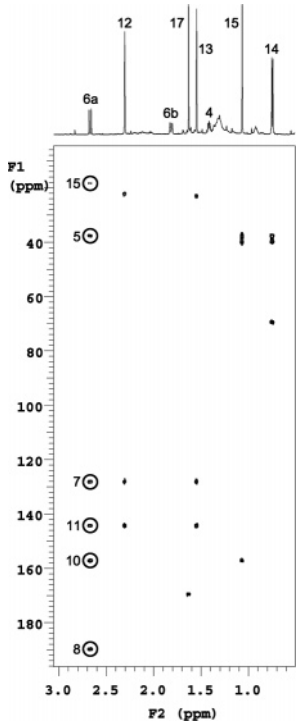


Figure 1. Partial gHMBC spectrum of **3** (599.9 MHz) with characteristic correlations encircled.

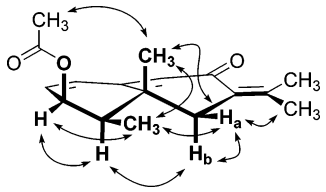


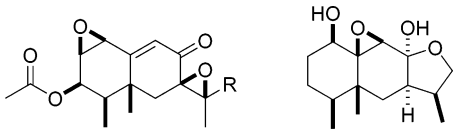
Figure 2. Configuration and conformation of **3** based on NOE.

general likeness to the ^{1}H NMR spectrum of PR toxin.[15,16] Confirmation of the eremophilane core structure was achieved by assignment of the spin patterns of H-1, 2, 3, 4, and 14 by gCOSY and 1D TOCSY experiments, linking the remaining ^{1}H NMR resonances by NOESY and 1D NOESY experiments (mix = 1.5 s), and finally deducing the chemical shifts of the carbon atoms by one-bond and long-range C−H correlations. In particular, the long-range C−H correlations of H-6a were very characteristic for **1**−**3** and revealed the chemical shifts of quaternary carbons C-5, 7, 8, 10, and 11 (Figure 1). Throughout the elucidation process, it proved useful to simulate the ^{13}C NMR spectra of hypothetical compounds for comparison with experimental data.

Given the rigid conformation of **3**, it was possible to assign its relative configuration by NOE correlations (Figure 2). The absolute configuration was inferred from the argument that **3** is a likely precursor in the stereochemically defined PR toxin biosynthetic pathway in *P. roqueforti*.[16] The initial assumption that **1** was the nonacetylated variant of **2** was only refuted upon inspection of the gHMBC spectrum, in which correlations of H-14 to C-3 (24.88 ppm) and H-9 to C-1 (72.13 ppm) were observed in lieu of the H-14 to C-3 (72.68 ppm) observed for **2** (Table 1). A literature search revealed that **2** was a known compound with ^{1}H and ^{13}C NMR data in agreement with the assignment.[13c] Furthermore, **1** contained minor impurities and was found to be a 1:2 mixture of 1α/β epimers, previously described as natural products.[12] The presence of hydroxyl epimers as metabolites of fungal biosynthesis has previously been observed in *Fusarium*, for which the ratios of diastereomers were specific for the individual species.[17] Finally, the samples were quantitated by NMR in an HTS-compatible solution of DMSO-d_6 with internal standard.

Table 1. Long-Range J_{CH} Correlations from (g)HMBC for Compounds **1**−**3**

position	1	2	3
1	n.d.	2, 10	2, 3, 5, 9, 10
2	indiscriminate	n.d.	1, 4, 10
3	indiscriminate	n.d.	1, 5, 16
4	n.d.	15	5, 14, 15
6	5, 7, 8, 10, 11, 15	5, 7, 8, 10, 11, 15	5, 7, 8, 10, 11, 15
9	1, 5, 7	1, 5, 7	1, 5, 7
12	7, 11, 13	7, 11, 13	7, 11, 13
13	7, 11, 12	7, 11, 12	7, 11, 12
14	1, 4, 5, 6	3, 4, 5, 6	3, 4, 5
15	4, 5, 6, 10	4, 5, 6, 10	4, 5, 6, 10
17	−	16	16



4 R = CH_3
5 R = CHO
6

Figure 3. Related fungal metabolites eremofortin A (**4**), PR toxin (**5**), and cuspidatol (**6**).

This strain of *P. roqueforti* is a potentially rich fermentable source of eremophilane sesquiterpenes, a class of compounds that have previously attracted some attention as core structures of inhibitors of HIV-1 integrase and HIV-1 Tat transactivation.[18,19] Several studies of *P. roqueforti* have probed the biogenesis and toxicity of PR toxin (**5**).[20] In this regard, we propose that **3** is a precursor of eremofortin A (**4**), and thus **5**. As a matter of speculation, a similar oxidative pathway could possibly convert **1** to the immunosuppressant cuspidatol (**6**), previously isolated from the endophytic fungus *Trichothecium roseum*.[21] The oxidative pathway leading to the formation of **5** requires oxygen, which emphasizes the need to maintain anaerobic conditions during the fermentation of silage for animal feed.[20e] The production of **5** has also been shown to be substrate dependent and especially enhanced by the presence of corn.[20e]

Superconducting magnet and cryogenic probe technologies have advanced the art of NMR to the state where microgram quantities can be sufficient for structure elucidations. LC-MS-SPE/NMR proved itself as a valuable tool to isolate and analyze small quantities of metabolites directly from crude extracts, but it also has the potential to produce enough quantitated material of selected metabolites for HTS bioassays (in vitro enzyme and receptor assays) and thus support most early phases of natural products research.

Experimental Section

General Experimental Procedures. NMR spectra were acquired on Varian Inova 600 spectrometers equipped with 5 mm HCN PFG Chili-Probes (cryogenic) and operating at 599.6 and 599.9 MHz for ^{1}H NMR. ^{13}C NMR data were acquired by ^{1}H observed (g)HSQC and (g)HMBC experiments (150.8 and 150.9 MHz equivalent). Deuterated solvents of "100%" grade from C/D/N Isotopes and 3 mm (335-PP) NMR tubes from Wilmad were used. ^{1}H and ^{13}C chemical shifts were referenced to the residual solvent peak (δ 7.16 ppm/128.39 ppm for C_6D_6). ACD/CNMR Predictor v. 8.09 software was used for simulation of ^{13}C NMR spectra. HRESIMS data were acquired on a Micromass Q-Tof Ultima time-of-flight mass spectrometer by direct infusion of isolated samples diluted with MeCN/H_2O (1:1) + 0.1% HCOOH. The LC-MS-SPE system consisted of a Waters Alliance 2795 HPLC, Knauer WellChrom K-120 solvent pump with an Alltech Elite degassing system (postcolumn dilution), Accurate ICP-04-20 flow-splitter by LC Packings, Bruker Esquire 4000 ion trap mass spectrometer, Waters 996 photodiode array detector, Bruker/Spark Prospekt II LC-SPE-NMR interface module with N_2-blanketing of the SPE compartment, and a Waters 2487 dual-wavelength absorbance detector for post-SPE monitoring, all managed by Bruker HyStar/EsquireControl software. Separation was achieved on a Phenomenex Synergi Max-RP (C12; 4

μm) 250 × 4.6 mm HPLC column with a gradient of MeCN/20 mM $NH_4OAc_{(aq)}$ (0.5 mL/min; 30:70 to 90:10 over 40 min; nondeuterated solvents). A postcolumn makeup flow of H_2O (1.5 mL/min) was added to reduce the solvent strength for trapping on Spark Hysphere Resin GP 10 × 2 mm SPE cartridges. The samples were filtered through 0.45 μm GHP Acrodisc (13 mm) syringe filters prior to injections.

Biological Material. *Penicillium roqueforti* DAOM 232127 (Dept. of Agriculture Ottawa Mycological Collection) was collected from grass silage from a dairy farm in Québec, where it was associated with ill thrift of cows. Details of the origin and macromorphological identification were previously decribed.[10] The isolate was cultivated in batches of 200 mL Czapek yeast extract medium (YES) in 500 mL flasks as both sedentary (2 batches) and shake cultures (2 batches) for 14 days.

Extraction and Isolation. Cultures were harvested by filtration, and the mycelium and broths were extracted as previously described.[11] Extracts were finally evaporated in vacuo and kept in the dark. The extracts of the broth and mycelium were dissolved in MeCN, combined, and distributed into four identical samples (500 μL ea.), representing the total metabolite production of the isolate under more or less aerobic conditions. The samples were diluted 10-fold with MeCN/2 mM $NH_4OAc_{(aq)}$, 1:1, and filtered, and injections of 100 μL were used for separation and isolation on the LC-MS-SPE system. By monitoring of UV and MS, peaks of interest were trapped on SPE cartridges either manually or as set by automation parameters. Double trapping (two injections/HPLC isolations; one SPE cartridge) afforded **1** and **2**, while **3** was the result of a single trapping. The SPE cartridges were dried with a constant flow of N_2 for 30 min each. The compounds were eluted off of the SPE cartridges directly into 3 mm NMR tubes with 170 μL of CD_3CN. Solvent changes were done in situ by evaporation in the NMR tubes by N_2-flow and subsequent reconstitution in 170 μL of C_6D_6 for optimized NMR. Final HTS-compatible solutions were prepared in 200 μL of DMSO-d_6 with 0.125 mM 1,4-dioxane internal standard for quantitation and storage.

1-Hydroxyeremophil-7(11),9(10)-dien-8-one (1): 1:2 mixture of 1α/β epimers; 25 μg (0.54 mM) in 200 μL of DMSO-d_6 with 0.125 mM 1,4-dioxane internal standard; ^{1}H NMR (C_6D_6, 599.9 MHz) δ 5.78 (1H, s, H-9), 5.63 (minor, s, H-9), 3.76 (1H, m, H-1), 3.71 (minor, m, H-1), 2.70 (1H, d, J = 13.6 Hz, H-6a), 2.29 (3H, s, H-12), 1.90 (1H, d, J = 13.6 Hz, H-6b), 1.74 (1H, m, H-2a), 1.74 (1H, m, H-3a), 1.55 (3H, s, H-13), 1.26 (1H, m, H-2b), 1.09 (1H, m, H-4), 1.07 (1H, m, H-3b), 1.05 (3H, s, H-15), 0.73 (3H, d, J = 6.7 Hz, H-14), 0.67 (1H, m, OH-1), 0.63 (minor, d, J = 6.5 Hz, H-14); ^{13}C NMR (C_6D_6, 150.9 MHz) δ 190.92 (C, C-8), 164.05 (C, C-10), 142.38 (C, C-11), 129.03 (CH, C-9), 128.39 (C, C-7), 126.43 (minor, CH, C-9), 72.62 (minor, CH, C-1), 72.13 (CH, C-1), 42.24 (CH_2, C-6), 42.22 (CH, C-4), 40.87 (C, C-5), 32.71 (CH_2, C-2), 24.88 (CH_2, C-3), 22.49 (CH_3, C-12), 21.80 (CH_3, C-13), 17.80 (CH_3, C-15), 15.23 (CH_3, C-14), 14.74 (minor, CH_3, C-14); HRESIMS *m*/*z* 235.1694 (calcd for $C_{15}H_{23}O_2$, 235.1698).

(3*S*)-3-Acetoxyeremophil-7(11),9(10)-dien-8-one (2): 7 μg (0.12 mM) in 200 μL of DMSO-d_6 with 0.125 mM 1,4-dioxane internal standard; ^{1}H NMR (C_6D_6, 599.6 MHz) δ 5.90 (1H, s, H-9), 4.93 (1H, m, J = 3.2 Hz, H-3), 2.66 (1H, d, J = 13.6 Hz, H-6a), 2.27 (3H, s, H-12), 2.24 (1H, m, J = 14.5 Hz, H-1a), 1.89 (1H, m, J = 14.5 Hz, H-2a), 1.81 (1H, d, J = 13.6 Hz, H-6b), 1.75 (1H, m, J = 14.5 Hz, H-2b), 1.66 (1H, m, J = 14.5 Hz, H-1b), 1.65 (3H, s, H-17), 1.54 (3H, s, H-13), 1.12 (1H, dd, J = 7.2, 3.3 Hz, H-4), 1.01 (3H, s, H-15), 0.73 (3H, d, J = 7.1 Hz, H-14); ^{13}C NMR (C_6D_6, 150.8 MHz) δ 190.05 (C, C-8), 169.20 (C, C-16), 164.96 (C, C-10), 141.96 (C, C-11), 127.85 (C, C-7), 126.82 (CH, C-9), 72.68 (CH, C-3), 43.95 (CH, C-4), 41.56 (CH_2, C-6), 40.91 (C, C-5), 30.41 (CH_2, C-2), 26.77 (CH_2, C-1), 22.60 (CH_3, C-12), 21.57 (CH_3, C-13), 20.36 (CH_3, C-17), 18.07 (CH_3, C-15), 11.14 (CH_3, C-14); HRESIMS *m*/*z* 277.1825 (calcd for $C_{17}H_{25}O_3$, 277.1804).

(3*S*)-3-Acetoxyeremophil-1(2),7(11),9(10)-trien-8-one (3): 19 μg (0.35 mM) in 200 μL of DMSO-d_6 with 0.125 mM 1,4-dioxane internal standard; ^{1}H NMR (C_6D_6, 599.9 MHz) δ 5.93 (1H, dd, J = 9.7, 5.4 Hz, H-2), 5.87 (1H, s, H-9), 5.79 (1H, d, J = 9.7 Hz, H-1), 5.25 (1H, t, J = 5.0 Hz, H-3), 2.67 (1H, d, J = 13.7 Hz, H-6a), 2.30 (3H, s, H-12), 1.81 (1H, d, J = 13.7 Hz, H-6b), 1.65 (3H, s, H-17), 1.54 (3H, d, J = 1.4 Hz, H-13), 1.44 (1H, dd, J = 7.2, 5.0 Hz, H-4), 1.06 (3H, s, H-15), 0.75 (3H, d, J = 7.2 Hz, H-14); ^{13}C NMR (C_6D_6, 150.9 MHz) δ 189.84 (C, C-8), 169.49 (C, C-16), 157.24 (C, C-10), 144.32 (C, C-11), 131.04 (CH, C-2), 130.75 (CH, C-1), 128.73 (CH, C-9), 128.17 (C, C-7), 69.41 (CH, C-3), 39.90 (CH, C-4), 39.69 (CH_2, C-6), 37.49 (C, C-5), 22.92 (CH_3, C-12), 22.07 (CH_3, C-13), 20.15 (CH_3, C-17), 18.38 (CH_3, C-15), 10.01 (CH_3, C-14); HRESIMS *m*/*z* 275.1643 (calcd for $C_{17}H_{23}O_3$, 275.1647).

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Supporting Information Available: HPLC chromatogram with trapping windows and extracted UV spectra and ^{1}H, (g)HSQC, (g)-HMBC, gCOSY, and NOESY NMR spectra for **1**, **2**, and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Newman, D. J.; Cragg, G. M.; Snader, K. M. *Nat. Prod. Rep.* **2000**, *17*, 215−234.
(2) MacCoss, M.; Baillie, T. A. *Science* **2004**, *303*, 1810−1813.
(3) Breinbauer, R.; Vetter, I. R.; Waldmann, H. *Angew. Chem., Int. Ed.* **2002**, *41*, 2878−2890.
(4) Newman, D. J.; Cragg, G. M.; Snader, K. M. *J. Nat. Prod.* **2003**, *66*, 1022−1037.
(5) Butler, M. S. *Nat. Prod. Rep.* **2005**, *22*, 162−195.
(6) Shu, Y.-Z. *J. Nat Prod.* **1998**, *61*, 1053−1071.
(7) Koehn, F. E.; Carter, G. T. *Nat. Rev. Drug Discovery* **2005**, *4*, 206−220.
(8) (a) Exarchou, V.; Godejohann, M.; Van Beek, T. A.; Gerothanassis, I. P.; Vervoort, J. *Anal. Chem.* **2003**, *75*, 6288−6294. (b) Godejohann, M.; Tseng, L.-H.; Braumann, U.; Fuchser, J.; Spraul, M. *J. Chromatogr. A* **2004**, *1058*, 191−196. (c) Krauser, J. A.; Voehler, M.; Tseng, L.-H.; Schefer, A. B.; Godejohann, M.; Guengerich, F. P. *Eur. J. Biochem.* **2004**, *271*, 3962−3969. (d) Seger, C.; Godejohann, M.; Tseng, L.-H.; Spraul, M.; Girtler, A.; Sturm, S.; Stuppner, H. *Anal. Chem.* **2005**, *77*, 878−885. (e) Clarkson, C.; Stærk, D.; Hansen, S. H.; Jaroszewski, J. *Anal. Chem.* **2005**, *77*, 3547−3553. (f) Miliauskas, G.; van Beek, T. A.; De Waard, P.; Venskutonis, R. P.; Sudhölter, E. J. R. *J. Nat. Prod.* **2005**, *68*, 168−172. (g) Lambert, M.; Stærk, D.; Hansen, S. H.; Sairafianpour, M.; Jaroszewski, J. *J. Nat. Prod.* **2005**, *68*, 1500−1509. (h) Clarkson, C.; Stærk, D.; Hansen, S. H.; Jaroszewski, J. *J. Nat. Prod.* **2006**, *69*, 527−530.
(9) Larsen, T. O.; Smedsgaard, J.; Nielsen, K. F.; Hansen, M. E.; Frisvad, J. C. *Nat. Prod. Rep.* **2005**, *22*, 672−695.
(10) Sumarah, M. W.; Miller, J. D.; Blackwell, B. A. *Mycopathologia* **2005**, *159*, 571−577.
(11) Nielsen, K. F.; Sumarah, M. W.; Frisvad, J. C.; Miller, J. D. *J. Agric. Food Chem.* **2006**, *54*, 3756−3763 (correction p 5216).
(12) (a) Bohlmann, F.; Knoll, K. H. *Liebigs Ann. Chem.* **1979**, 470−472. (b) Hagiwara, H.; Uda, H.; Kodama, T. *J. Chem. Soc., Perkin Trans. 1* **1980**, 963−977. (c) Ahmed, A. A. *J. Nat. Prod.* **1991**, *54*, 271−272.
(13) (a) Brooks, C. J. W.; Keates, R. A. B. *Phytochemistry* **1972**, *11*, 3235−3245. (b) Takagi, I.; Tazuke, Y.; Naya, K. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 3320−3323. (c) Torii, S.; Inokuchi, T.; Kawai, K. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 861−866.
(14) Nielsen, K. F.; Smedsgaard, J. *J. Chromatogr., A* **2003**, *1002*, 111−136.
(15) Wei, R. D.; Schnoes, H. K.; Hart, P. A.; Strong, F. M. *Tetrahedron* **1975**, *31*, 109−114.
(16) Moreau, S.; Biguet, J.; Lablache-Combier, A.; Baert, F.; Foulon, M.; Delfosse, C. *Tetrahedron* **1980**, *36*, 2989−2997.
(17) Greenhalgh, R.; Fielder, D. A.; Morrison, L. A.; Charland, J.-P.; Blackwell, B. A.; Savard, M. E., ApSimon, J. W. *J. Agric. Food Chem.* **1989**, *37*, 699−705.
(18) Singh, S. B.; Zink, D.; Polishook, J.; Valentino, D.; Shafiee, A.; Silverman, K.; Felock, P.; Teran, A.; Vilella, D.; Hazuda, D. J.; Lingham, R. B. *Tetrahedron Lett.* **1999**, *40*, 8775−8779.
(19) Jayasuriya, H.; Zink, D. L.; Polishook, J. D.; Bills, G. F.; Dombrowski, A. W.; Genilloud, O.; Pelaez, F. F.; Herranz, L.; Quamina, D.; Lingham, R. B.; Danzeizen, R.; Graham, P. L.; Tomassini, J. E.; Singh, S. B. *Chem. Biodiversity* **2005**, *2*, 112−122.
(20) (a) Polonelli, L.; Morace, G.; Delle Monache, F.; Samson, R. A. *Mycopathologia* **1978**, *66*, 99−104. (b) Moreau, S.; Lablache-Combier, A.; Biguet. J. *Appl. Environ. Microbiol.* **1980**, *39*, 770−776. (c) Chen, F.-C.; Chen, C.-F.; Wei, R.-D. *Toxicon* **1982**, *20*, 433−441. (d) Chang, S.-C.; Wei, Y.-H.; Liu, M.-L.; Wei, R.-D. *Appl. Environ. Microbiol.* **1985**, *49*, 1455−1460. (e) Chang, S.-C.; Wei, Y.-H.; Wei, D.-L.; Chen, Y.-Y.; Jong, S.-C. *Appl. Environ. Microbiol.* **1991**, *57*, 2581−2585.
(21) Kawamura, H.; Pulici, M.; Koshino, H.; Esumi, Y.; Uzawa, J.; Kumagai, H.; Sugawara, F. *Nat. Prod. Lett.* **2000**, *14*, 299−304.

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