Notes

Decipinin A and Decipienolides A and B: New Bioactive Metabolites from the Coprophilous Fungus *Podospora decipiens*

Yongsheng Che,[†] James B. Gloer,*[,†] Brenda Koster,[‡] and David Malloch[‡]

Department of Chemistry, University of Iowa, Iowa City, Iowa, 52242, and Department of Botany, University of Toronto, Toronto, Ontario, M5S 1A1, Canada

Received November 16, 2001

Decipinin A (**1**), a new compound with antifungal and antibacterial activity, has been isolated from liquid cultures of the coprophilous fungus *Podospora decipiens* (JS 270). Two new tetracyclic sesquiterpene lactones, decipienolides A (**2**) and B (**3**), were also obtained from this isolate as an inseparable mixture of epimers that showed antibacterial activity. The structures of **1**−**3** were elucidated by analysis of 1D and 2D NMR data, aided by chemical shift comparisons to related compounds.

Chemical investigations of coprophilous fungi in our laboratory have resulted in the discovery of a variety of novel antifungal compounds.[1] Our prior studies of coprophilous isolates from the genus *Podospora* have led to the isolation of several new antifungal agents.[2−4] As part of this ongoing program, an isolate of *Podospora decipiens* (Lasiosphaeriaceae) was chemically investigated, and this study led to the isolation of a new antifungal polyketide-derived metabolite that we named decipinin A (**1**). To our knowledge, only one other metabolite containing the ring system found in **1** has been previously reported.[5] Two new tetracyclic sesquiterpenoids (decipienolides A and B; **2** and **3**) were also obtained from this isolate as an inseparable epimeric mixture. Decipienolides A and B are closely related to the expansolides[6] and represent a second rarely encountered ring system. This account describes the isolation, structure elucidation, and biological activities of compounds **1**−**3** and constitutes only the second report of chemistry from *P. decipiens*. The antifungal agent podosporin A was obtained from a different isolate of *P. decipiens* in earlier work from our laboratory[4] and was among the first novel compounds encountered in our studies of coprophilous fungi.

The EtOAc extract of liquid cultures of an isolate of *P. decipiens* (JS 270) exhibited antifungal and antibacterial effects and was subjected to silica gel vacuum liquid chromatography (VLC) and column chromatography to afford decipinin A (**1**). Analysis of the ^{1}H and ^{13}C NMR data for compound **1** revealed the presence of four methyl groups, six aliphatic methylene units, three oxygenated sp^3 methine units, one mono-oxygenated quaternary carbon, one ketal carbon (δ_C 101.1), 10 olefinic carbons (eight protonated), four carboxyl carbons, and two α,β-unsaturated ketone carbons. On the basis of these data and HRFABMS analysis, the molecular formula of decipinin A (**1**) was determined to be $C_{31}H_{36}O_{12}$ (14 degrees of unsaturation). DEPT data were consistent with the inclusion of a single exchangeable proton.

Table 1. NMR Data for Decipinin A (**1**) in $CDCl_3$

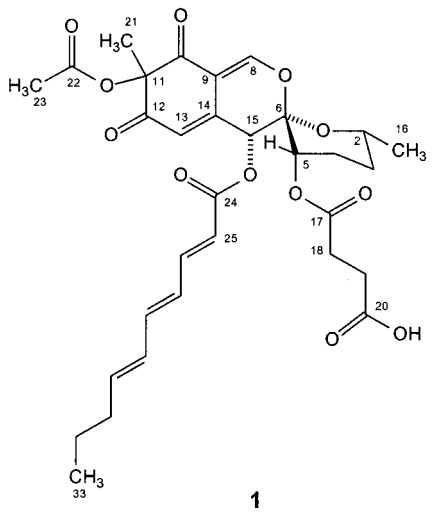
position	δ_H (mult., J_{HH})	δ_C	HMBC (H→C#)
1			
2	3.85 (ddq, 2.5, 12, 6.0)	69.1	
3_{ax}	1.59 (dddd, 15, 15, 12, 3.6)	26.2	
3_{eq}	1.52 (m)		
4_{ax}	2.12 (br q, 14)	23.3	2
4_{eq}	1.92 (br dq, 14, 3)		2, 6
5	5.07 (br t, 2.7)	65.2	3, 6, 17
6		101.1	
7			
8	7.63 (d, 1.2)	154.1	6, 9, 10, 14
9		110.8	
10		192.1	
11		84.8	
12		194.0	
13	6.16 (d, 1.2)	122.3	9, 11, 15
14		141.9	
15	5.82 (s)	64.3	6, 9, 13, 14, 24
16	1.11 (d, 6.0)	21.2	2, 3
17		170.5	
18	2.64 (m)[a]	28.7[a]	17, 19, 20
19	2.61 (m)[a]	28.9[a]	17, 18, 20
20		177.2	
21	1.49 (s)	22.2	10, 11, 12
22		169.7	
23	2.11 (s)	19.9	22
24		165.1	
25	5.67 (d, 15)	117.5	24, 27
26	7.18 (dd, 15, 11)	147.2	24, 25, 27, 28
27	6.12 (dd, 15, 11)	127.4	25, 26, 28, 29
28	6.51 (dd, 15, 11)	142.8	26, 29, 30
29	6.09 (dd, 15, 11)	129.9	27, 28, 30, 31
30	5.92 (dt, 15, 7.2)	141.4	28, 31, 32
31	2.09 (br q, 7.2)	35.0	29, 30, 32, 33
32	1.40 (sextet, 7.2)	22.1	30, 31, 33
33	0.88 (t, 7.2)	13.6	31, 32

[a] These assignments and the corresponding HMBC correlations may be interchanged.

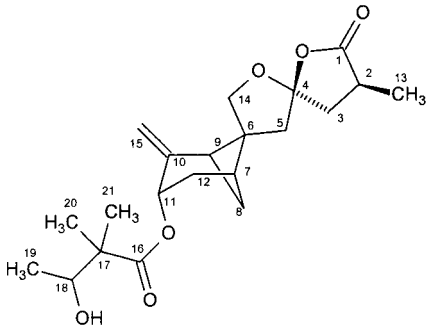
The NMR data (Table 1) revealed the presence of an acetate unit (δ_H 2.11, δ_C 19.9/169.7), and this was confirmed by an HMBC correlation of H_3-23 to C-22. Three isolated proton spin systems corresponding to the C2−C5/C16, C18−C19, and C25−C33 subunits of structure **1** were established on the basis of COSY data and supported by analysis of HMBC data (Table 1). HMBC correlations of

* To whom correspondence should be addressed. Tel: 319-335-1361. Fax: 319-335-1270. E-mail: james-gloer@uiowa.edu.
[†] University of Iowa.
[‡] University of Toronto.

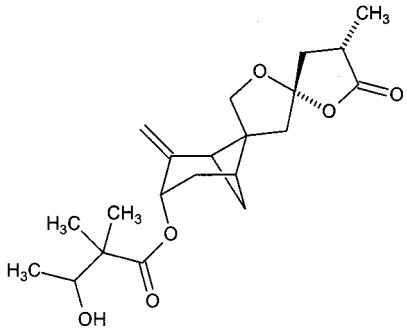
10.1021/np010575p CCC: $22.00
Published on Web 05/03/2002



1



2



3

the mutually coupled H_2-18 and H_2-19 signals to both C-17 (δ_C 170.5) and C-20 (δ_C 177.2) established the presence of a succinyl moiety. HMBC correlations of H-25 and H-26 to carboxyl carbon C-24 (δ_C 165.1) enabled identification of a 2,4,6-decatrienoic acid subunit. All three double bonds in this unit were assigned the *E*-geometry on the basis of the *J*-values for the olefinic protons (ca. 15 Hz in each case). The observation of long-range coupling between H-8 and H-13 revealed that the C8−C9 and C13−C14 double bonds are conjugated to each other, and this was confirmed by HMBC correlations of H-8 with C-9 and C-14, as well as a correlation of H-13 to C-9.

HMBC correlations of H_3-21 to α,β-unsaturated ketone carbons C-10 (δ_C 192.1) and C-12 (δ_C 194.0), as well as to oxygenated quaternary carbon C-11 (δ_C 84.8), located the methylated, oxygenated quaternary carbon C-11 between the two ketone carbonyl groups. HMBC correlations of H-8 to C-10 and of H-13 to C-11 enabled construction of the C8−C14 unit, thus completing the cyclohexenedione ring. HMBC correlations of H-15 to C-9, C-13, C-14, and ketal carbon C-6, along with correlations of H-5 and H-8 to C-6, led to the connection of C-5 and C-15 to C-6, and the connection of C-15 to C-14. These data, together with the downfield chemical shift of C-8 (δ_C 154.1), also indicated that C-6 and C-8 are connected to the same oxygen atom, thereby completing the second six-membered ring. HMBC correlations of H-5 to C-17 and of H-15 to C-24 enabled location of the succinyl and 2,4,6-decatrienoyl units at the C-5 and C-15 positions. The only exchangeable proton in decipinin A (**1**) was assigned to a free carboxylic acid group in the succinyl unit. This is consistent with the fact that decipinin A did not react with acetic anhydride and suggested by default that C-2 and C-6 must be attached to the same oxygenated atom to complete the third six-membered ring. Geometric constraints would preclude linkage of either C-2 or C-6 to the oxygen at position 11. Thus, the acetate group must be attached to C-11 to complete the structure of **1** as shown. Location of the acetate group at C-11 is also consistent with the absence of any HMBC correlations for C-22 aside from that observed with H_3-21.

The relative stereochemistry of decipinin A (**1**) was proposed on the basis of NOESY data and $^1H-^1H$ coupling constants. The *trans*-diaxial coupling constant observed for H-2 (12 Hz) indicated that CH_3-16 adopts an equatorial orientation. The H-5 signal appeared as a broad triplet with a small coupling constant (2.7 Hz), indicating that it must be equatorial. A NOESY correlation of H-5 to H-15 required C-5 and H-15 to be *cis* with respect to the dihydropyran ring. These data establish the relative stereochemistry of all the stereocenters in decipinin A except C-11. Unfortunately, no relevant NOESY correlations were observed for this center, and it is quite remote from the other stereocenters in the molecule. Thus, the stereochemistry at C-11 was not assigned.

The core structure of decipinin A (**1**) resembles the azaphilone class of fungal metabolites, which includes sclerotiorin[7] and austdiol.[8] However, decipinin A differs from most known members of this class in that it contains a third ring spiro-fused with the azaphilone core at C-6. The only precedent for such a ring system is daldinin C,[5] a metabolite of the fungus *Daldinia concentrica*. As expected, the ^1H and ^{13}C NMR data for the core structure of decipinin A (**1**) closely match those reported for daldinin C.

Decipienolides A and B (**2** and **3**) were obtained as an inseparable mixture of epimers in a 6:4 ratio. Exhaustive efforts to separate the mixture using column chromatography and HPLC employing different stationary and mobile phases were unsuccessful in even partially resolving the epimers. Thus, structure elucidation was performed on the mixture.

The molecular formula for both decipienolides A (**2**) and B (**3**) was determined to be $C_{21}H_{30}O_6$ (seven degrees of unsaturation) by analysis of HRFABMS and NMR data (Table 2). Analysis of ^1H and ^{13}C NMR data for the **2/3** mixture revealed the presence in each component of four methyl groups, five methylene units (one oxygenated), five methine units (two oxygenated), three nonprotonated sp^3 carbons (including one ketal carbon), a 1,1-disubstituted olefin, and two carboxyl carbons. DEPT data and the molecular formula required the presence of one exchangeable proton. Consideration of all of these data led to the conclusion that decipienolides A (**2**) and B (**3**) are tetracyclic.

The NMR data for the major and minor epimers were considered separately. In the major isomer, three isolated

Table 2. NMR Data for Decipienolides A (**2**) and B (**3**) in $CDCl_3$

	decipienolide A (**2**)			decipienolide B (**3**)	
no.	δ_H (mult., J_{HH})	δ_C	HMBC (H# → C#)	δ_H (mult., J_{HH})	δ_C
1		178.6			178.2
2	2.90 (m)	34.7	1, 3, 13	2.71 (m)	35.6
3a	2.47 (dd, 13, 8.4)	39.8	1, 2, 4, 5, 12	2.50 (dd, 13, 9.0)	39.0
3b	1.98 (dd, 13, 11)		1, 2, 4, 5, 12	2.04 (dd, 13, 6.0)	
4		113.7			115.0
5a	2.63 (d, 13)	47.0	3, 4, 6, 7, 9	2.66 (d, 13)	47.6
5b	2.27 (d, 13)		3, 4, 6, 7, 9	2.20 (d, 13)	
6		50.6			50.6
7	2.56 (m)	39.9		2.27 (m)	40.5
8a	2.30 (m)	29.4	6, 7, 9, 10, 14	2.30 (m)	29.5
8b	1.70 (d, 9.6)		6, 7, 9, 10, 14	1.70 (d, 9.6)	
9	2.77 (d, 5.6, 5.4)	51.6	5, 7, 8, 10, 11, 15	3.08 (dd, 5.6, 5.4)	50.6
10		147.9			146.7
11	5.47 (d, 7.2)	67.6	7, 9, 10, 12, 15, 16	5.44 (d, 6.6)	67.7
12a	2.39 (m)	32.7	7, 8	2.33 (m)	33.4
12b	1.84 (m)		7, 8	1.84 (m)	
13	1.25 (d, 7.2)	14.9	1, 2, 3	1.33 (d, 7.2)	16.5
14a	3.77 (d, 9.6)	71.3	4, 5, 6, 7, 9	3.76 (d, 9.6)	71.8
14b	3.61 (d, 9.6)		4, 5, 6, 7, 9	3.62 (d, 9.6)	
15a	5.10 (br s)	115.4	9, 10, 11	5.13 (br s)	116.3
15b	4.97 (br s)		9, 10, 11	5.09 (br s)	
16		176.7			176.7
17		46.6			46.6
18	3.86 (m)	72.2	16, 17, 19, 20, 21	3.86 (m)	72.2
19	1.12 (d, 6.6)	17.4	17, 18	1.12 (d, 6.6)	17.4
20	1.15 (s)	21.9	16, 17, 18, 21	1.15 (s)	21.9
21	1.13 (s)	19.4	16, 17, 18, 20	1.13 (s)	19.4

proton spin systems corresponding to the C2/C3/C13, C18/C19, and C7−C12 (including the C10/C15 olefin) subunits of structure **2** were established on the basis of COSY data and confirmed by HMBC correlations. HMBC correlations of H_3-13 to carboxyl carbon C-1 (δ_C 178.6), and of H_2-3 to ketal carbon C-4 (δ_C 113.7) and to C-5 (δ_C 47.0), completed the partial structure from C-1 to C-5. HMBC correlations of H_2-5 and isolated oxymethylene H_2-14 with C-6, C-7, and C-9, together with correlations of H-7 and H-9 to C-6, required linkage of C-6 to C-5, C-14, C-7, and C-9. A key HMBC correlation between H_2-14 and C-4 completed the tetrahydrofuran ring. An IR absorption at 1774 cm^{-1} suggested the presence of a γ-lactone, which could be accounted for by connection of the other C-4 oxygen atom to C-1.

HMBC cross-peaks of the H_3-19 doublet with quaternary carbon C-17 (δ_C 46.6) and the oxygenated methine C-18 (δ_C 72.3) revealed that C-17 must be linked to C-18. HMBC correlations of H_3-20 and H_3-21 to C-17, C-18, and the second carbonyl carbon (C-16; δ_C 176.7) were also observed. On the basis of these data, a 3-hydroxy-2,2-dimethylbutyryl moiety was identified. This unit must acylate the oxygen atom at C-11 by virtue of an HMBC correlation between H-11 and C-16. Therefore, the gross structure of the major isomer (decipienolide A) was established as shown in **2**.

An unusually strong $^4J_{CH}$ correlation between one of the C-8 protons and C-14 was observed. Long-range *W*-type couplings are not uncommon in rigid, strained ring systems,[9,10] and observation of a heteronuclear coupling of this type is fully consistent with the well-documented occurrence of a strong four-bond homonuclear coupling between protons that have an analogous disposition across a four-membered ring.[9,11]

The isomeric minor component decipienolide B (**3**) was assigned the same gross structure as **2** by analysis of the corresponding minor signals in the NMR data for the mixture (Table 2). A literature search led to a report of a similar mixture of isomeric sesquiterpene lactones called expansolides A and B[6] that possess the same ring system as decipienolides A and B. Expansolides A and B were reported as metabolites of *Penicillium expansum*, and the structures of these compounds were established by X-ray crystallography, along with NOE comparisons.[6] The spectral properties of decipienolides A and B are nearly identical to those of expansolides A and B, except for the signals associated with the acyl group attached to the oxygen atom at C-11. The structure assigned for the tetracyclic portion of **2** and **3** matches the corresponding portion of the expansolides. Moreover, expansolides A and B were similarly prone to equilibration, and the X-ray study reported for the expansolides was performed on a single crystal of a 1:1 mixture of the two epimers. The very close similarities in NMR chemical shifts and *J*-values[6] clearly indicated that the major component, decipienolide A (**2**), has the same relative stereochemistry as expansolide A, while decipienolide B (**3**) has the same relative stereochemistry as expansolide B. However, the relative stereochemistry at the additional stereocenter in the 3-hydroxy-2,2-dimethylbutyryl moiety (C-18) found in **2** and **3** was not determined, as it could not be related to that of the rest of the molecule. Degradation experiments were not pursued due to the limited quantity of material isolated, together with the expected complexity of chemical degradation mixtures.

Aside from the expansolides, the only other previously known compound that possesses the same tetracyclic ring system as **2** and **3** is massarinolin A, an antibacterial metabolite isolated in our laboratory from the freshwater fungus *Massarina tunicata*.[10] Interestingly, to our knowledge, the 3-hydroxy-2,2-dimethylbutyric acid side chain found in **2** and **3** has not been previously reported as a constituent of a natural product, although it has been isolated as a degradation product of atrovenetin and related fungal metabolites.[12]

Decipinin A (**1**) showed activity in a standard disk assay[13] against *Fusarium verticillioides* (ATCC 24378) at

250 μg/disk, affording an inhibitory zone of 22 mm. Compound **1** and the **2**/**3** mixture were active in standard disk assays against the Gram-positive bacterium *Bacillus subtilis* (ATCC 6051), affording zones of inhibition of 9−10 mm at 200 μg/disk. None of these compounds displayed activity against the competitor coprophilous fungi *Ascobolus furfuraceus* (NRRL 6460) and *Sordaria fimicola* (NRRL 6459), or against *Candida albicans* (ATCC 90029) at this level.

Experimental Section

General Procedures. ^{1}H, COSY, HMBC, HMQC, and NOESY NMR spectra were recorded using a Bruker AMX-600 spectrometer operating at a ^{1}H frequency of 600 MHz. ^{13}C NMR spectra were recorded using a Bruker AC-300 instrument at 75 MHz. Residual protiated solvent signals for $CDCl_3$ (δ 7.24/77.0) were used as internal references. FABMS and HRFABMS data were recorded with a VG ZAB-HF mass spectrometer. EIMS data were obtained using a VG Trio 1 instrument operating at 70 eV. Optical rotations were measured on a JASCO model DIP-1000 digital polarimeter, UV spectra were recorded on a HP 8452A diode array spectrophotometer, and IR data were obtained on a Mattson Cygnus 25 FTIR instrument.

Fungal Material. The isolate of *P. decipiens* (Wint. ex Fuckel) Niessl. employed in this study was obtained from a sample of sheep dung collected by J. A. Scott in South Australia on March 19, 1994. This isolate was assigned the accession number JS 270 in the D. Malloch culture collection at the University of Toronto. The subculture was inoculated (0.5 cm^2 agar plugs taken from stock cultures) into six 2 L Erlenmeyer flask, each containing 400 mL of autoclaved potato dextrose broth (Difco) in distilled water. Liquid cultures were incubated at room temperature on an orbital shaker operating at 150 rpm for 25 days.

Extraction and Isolation. The filtered culture broth (2.4 L) was extracted with EtOAc (4 × 500 mL), and the organic phase was dried over $MgSO_4$ and concentrated to afford 3.45 g of brown oil. The crude extract was subjected to silica gel VLC using a hexane−CH_2Cl_2−MeOH solvent gradient. The fractions that were eluted between 50:50 hexane−CH_2Cl_2 and 98:2 CH_2Cl_2−MeOH were combined (1.2 g) and subjected to silica gel column chromatography using the same solvent system to afford decipinin A (**1**; 35 mg, eluted with 90:10 CH_2Cl_2−hexane). The VLC fractions eluted with 99:1 CH_2Cl_2−MeOH were combined (390 mg) and subjected to Sephadex LH-20 column chromatography using a hexane−CH_2Cl_2−acetone step gradient. A portion of the initial fraction (132 mg; eluted with 1:4 hexane−CH_2Cl_2) was further separated by reversed-phase HPLC (Alltech HS Hyperprep 100 BDS C_{18}; 10 × 250 mm; 25%−45% CH_3CN in H_2O over 40 min, 45%−60% over 25 min, isocratic for 25 min, then 60%−100% over 20 min) to afford the decipienolides A and B mixture (**2** and **3**; 7.0 mg; t_R 98 min).

Decipinin A (1): white powder; mp 99−101 °C; $[\alpha]_D$ +3.2° (*c* 0.3, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 318 (ϵ 2800), 260 (ϵ 1500); IR (CH_2Cl_2) 3500−2700 (br, w), 3063, 2965, 2933, 2871, 1749, 1716, 1680, 1630, 1619, 1583, 1251, 1222 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS (70 eV) *m*/*z* 600 (M$^+$; rel int 1), 558 (0.3), 436 (0.8), 335 (2), 317 (1), 274 (13), 231 (17), 191 (8), 165 (6), 149 (100), 107 (78), 91 (19), 79 (27), 55 (82); FABMS (3-NBA) obsd *m*/*z* 601 ([M + H]$^+$; rel int 100), 559 (13), 501 (6), 435 (9), 400 (9), 335 (13), 275 (37), 247 (20), 191 (29), 179 (26), 165 (63); HRFABMS (PEG 600/NaI/3-NBA) obsd *m*/*z* 601.2285 (M + H)$^+$, calcd for $C_{31}H_{37}O_{12}$, 601.2283.

Decipienolides A (2) and B (3) (inseparable epimeric mixture): colorless oil; $[\alpha]_D$ −9.7° (*c* 0.3 CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 258 (ϵ 1900); IR (CH_2Cl_2) ν_{max} 2590, 2933, 1774, 1736, 1461, 1272, 1147 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; FABMS (3-NBA) obsd *m*/*z* 379 ([M + H]$^+$; rel int 100), 265 (33), 247 (42); HRFABMS (PEG 400/3-NBA/TFA) obsd *m*/*z* 379.2126 (M + H)$^+$, calcd for $C_{21}H_{31}O_6$, 379.2121.

Acknowledgment. We thank the National Institutes of Health (R01 AI 27436) for financial support.

References and Notes

(1) Gloer, J. B. In *The Mycota*; IV: Environmental and Microbial Relationships; Wicklow, D. T., Söderström, B. E., Eds.; Springer: New York, 1997; pp 249−268.
(2) Wang, H. J.; Gloer, K. B.; Gloer, J. B.; Scott, J. A.; Malloch, D. *J. Nat. Prod*. **1997**, *60*, 629−631.
(3) Wang, Y.; Gloer, J. B.; Scott, J. A.; Malloch, D. *J. Nat. Prod*. **1993**, *56*, 341−344.
(4) Weber, H. A.; Baenziger, N. C.; Gloer, J. B. *J. Org. Chem*. **1988**, *53*, 4567−4569.
(5) Hashimoto, T.; Tahara, S.; Takaoka, S.; Tori, M.; Asakawa, Y. *Chem. Pharm. Bull*. **1994**, *42*, 2397−99.
(6) Massias, M.; Rebuffat, S.; Molho, L.; Chiaroni, A.; Riche, C.; Bodo, B. *J. Am. Chem. Soc*. **1990**, *112*, 8112−8115.
(7) Whalley, W. B.; Ferguson, G.; Marsh, W. C.; Restivo, R. J. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1366−1369.
(8) Vleggaar, R.; Steyn, P. S.; Nagel, D. W. *J. Chem. Soc., Perkin Trans. 1* **1974**, 45−49.
(9) Sohár, P. *Nuclear Magnetic Resonance Spectroscopy*; CRC Press: Boca Raton, FL, 1983; Vol. I, pp 67−68.
(10) Oh, H.; Gloer, J. B.; Shearer, C. A. *J. Nat. Prod*. **1999**, *62*, 497−501.
(11) Pretsch, E.; Clerc, T.; Seibl, T.; Simon, W. *Tables of Spectral Data for Structure Determination of Organic Compounds*, 2nd ed.; Springer-Verlag: New York, 1989; p H190.
(12) Brooks, J. S.; Morrison, G. A. *J. Chem. Soc., Perkin Trans. 1* **1974**, *18*, 2114−19.
(13) Wicklow, D. T.; Joshi, B. K.; Gamble, W. R.; Gloer, J. B.; Dowd, P. F. *Appl. Environ. Microbiol*. **1998**, *64*, 4482−4484.

NP010575P