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Xylarenones C−E from an Endophytic Fungus Isolated from *Alibertia macrophylla*

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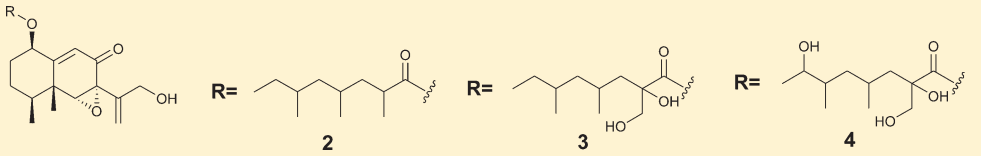
Supporting Information

ABSTRACT:



Xylarenones C−E (**2**−**4**), three new eremophilane sesquiterpenes, have been isolated from solid substrate cultures of a *Camarops*-like endophytic fungus isolated from *Alibertia macrophylla*. The structures were elucidated by analysis of spectroscopic data. Compounds were evaluated in subtilisin and pepsin protease assays, and compound **2** showed potent inhibitory activity against both proteases.

Both laboratory and field studies have demonstrated that wild plant species require fungal endophytes for stress tolerance and survival.[1] Therefore, each of the nearly 300 000 species of terrestrial plants on Earth may host one or more fungal endophytes.[2] Some endophyte−plant associations reportedly confer benefits to host plants, including tolerance to herbivory, heat, salinity, diseases, and drought.[1] Moreover, the production of bioactive secondary metabolites by fungal endophytes could in some cases enhance a plants ability to avoid microbial infections, overpredation, and space competition by other invasive plant species.[3] Thus, the study of fungal endophytes is currently considered a reasonable approach to the discovery of novel, bioactive natural products.[3,4]

Proteases are important enzymatic targets because these proteins control the formation of functional peptides that participate in physiological processes.[5] As part of our ongoing search for new protease inhibitors from endophytic fungi of the Cerrado and of the Atlantic Rain Forest of Brazil, four fungal strains were isolated from *Alibertia macrophylla* (Rubiaceae).[6] One strain (AM-02) yielded a crude extract with protease inhibitory activity and was chosen for chemical, biological, and taxonomic investigations. Herein we report the isolation of four eremophilane sesquiterpenes, three of which are new, and all of them displayed inhibitory activity toward pepsin. Compound **2** also inhibited subtilisin.

RESULTS AND DISCUSSION

The sesquiterpene xylarenone A (**1**) was identified by comparison of its IR, MS, ^{1}H NMR, and ^{13}C NMR data with literature values for the same compound previously isolated from *Xylaria* sp. NCY2. ($[\alpha]^{20}_D$ = +144.1, *c* 4.614, $CHCl_3$).[7]

Xylarenone C (**2**) was isolated as an optically active, colorless oil ($[\alpha]^{28}_D$ +14). Analysis by HRESIMS indicated a $[M + Na]^+$ ion at *m*/*z* 455.2773, corresponding to the formula $C_{26}H_{40}NaO_5$. The IR spectrum of **2** showed a broad band at 3442 cm^{-1} (ν_{O-H}), as well as bands assigned to ester (1730 cm^{-1}) and α,β-unsaturated carbonyl (1679 cm^{-1}) functionalities. Analysis of ^{13}C (BBD and DEPT 135) NMR spectra indicated the presence of six methyl, seven methylene, seven methine, and six nonprotonated carbons, two of which were assigned to carbonyl groups, at δ 195.2 (α,β-unsaturated ketone) and δ 175.0 (ester). The ^{1}H NMR spectrum of **2** showed the presence of a deshielded oxymethine proton at

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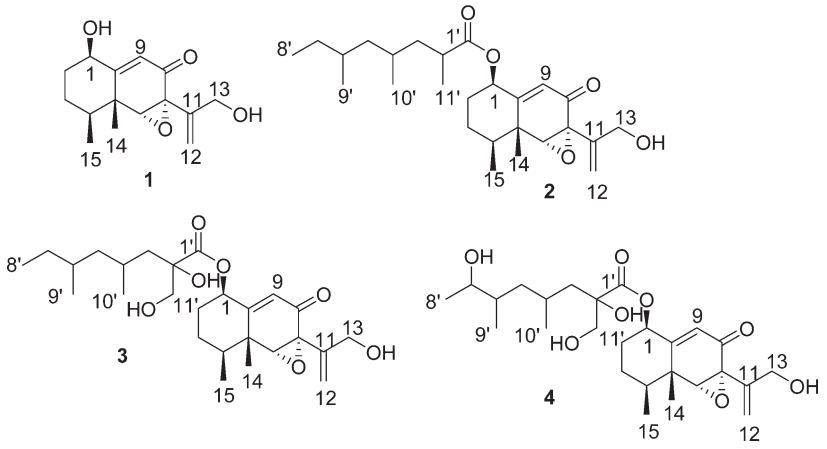

Figure 1. Structures of compounds **1**−**4** isolated from the *Camarops*-like endophyte. AM-02.

Table 1. ^{1}H NMR Data for Xylarenones C−E, 2−4 ($CDCl_3$, 500 MHz)

	2	3	4
position	δ_H (mult., *J* in Hz)	δ_H (mult., *J* in Hz)	δ_H (mult., *J* in Hz)
1	5.41 t (3.0)	5.49 dd (2.0, 2.0)	5.50 dd (2.0, 2.0)
2	1.94 m	1.56 m	1.50 m
3	1.86 m; 1.42 m	1.68 m	1.70 m
4	1.88 m	1.88 m	1.87 m
5			
6	3.32 s	3.33 s	3.32 s
7			
8			
9	5.95 s	6.0 s	6.0 s
10			
11			
12	5.25 s; 5.33 dd (1.5, 1.0)	5.24 s; 5.31 dd (1.5, 1.0)	5.29 s; 5.23 s
13	4.12 d (13.5); 4.27 d (13.5)	4.14 d (13.0); 4.28 d (13.0)	4.14 d (13.0); 4.28 d (13.0)
14	1.23 s	1.23 s	1.23 s
15	1.06 d (7.0)	1.08 d (7.0)	1.08 d (6.5)
1′			
2′	2.50 m		
3′	1.63 m	1.35 dd (14.0, 8.0); 1.60 dd (14.0, 4.0)	1.30 m; 1.38 m
4′	1.39 m	1.55 m	1.62 m
5′	1.29 m	0.83 m; 1.38 m	0.82 m; 0.84 m
6′	1.19 m	1.23 m	1.46 m
7′	1.35 m	1.28 m	3.52, dq (5.5, 6.5)
8′	0.77 t (6.5)	0.78 t (7.5)	1.03 d (6.5)
9′	0.75 d (6.5)	0.75 d (6.5)	0.76 d (7.0)
10′	0.77 d (6.5)	0.88 d (6.5)	0.94 d (6.5)
11′	1.08 d (7.0)	3.44 d (11.0); 3.60 d (11.0)	3.62 d (11.0); 3.46 d (11.0)

δ 5.41, assigned to H-1 by analysis of ^{1}H−^{1}H COSY and HMBC spectra, as well as by comparison with data obtained for xylarenone A (**1**).[6] The remaining ^{1}H and ^{13}C NMR signals assigned to the sesquiterpene moiety of **2** were practically identical to those of **1** (Table 1). The presence of a 2,4,6-trimethyloctanoic acid residue attached to the sesquiterpene skeleton was indicated by the presence of three methyl groups observed as doublets at δ 1.08, 0.77, and 0.75, along with a fourth methyl group as a triplet at δ 0.77. These methyl groups were interconnected via methine and methylene groups, the first of which at δ 2.50 (CH-2′, δ_C 37.9) attached to the saturated ester carbonyl (HMBC). A detailed analysis of ^{1}H−^{1}H COSY and HMBC spectra allowed us to assign the ^{1}H and ^{13}C chemical shifts to the sequentially connected methine and methylenes units and to attach each methyl group to the corresponding methine or methylene unit residue. The attachment position of the 2,4,6-trimethyloctanoic acid residue to the

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sesquiterpene moiety of **2** was defined on the basis of HMBC correlations between H-1 and C-1′, and between H-9 and C-1, and also by comparison of the CH-1 and CH_2-13 chemical shift values of compounds **1** and **2**, which indicated a significant change at CH-1, but not at CH_2-13. The relative configuration of the sesquiterpene moiety of **2** was established by comparison with data recorded for **1**, as well as by analysis of 1D-NOESY NMR spectra. Dipolar couplings were observed between H-1 at δ 5.41 and H-9 (δ 5.95) and between H-6 at δ 3.32 and Me-15 (δ 1.06) and Me-14 (δ 1.23). The (1*R**, 4*S**, 5*R**, 6*R**, 7*R**) relative stereochemistry is in agreement with the structure of **1** and with other fungal eremophilane sesquiterpenes.[9−11] The configurations at C-2′, C-4′, and C-6′ of the octanoate ester unit were not established. Other fungal eremophilane-type sesquiterpenes are known that also possess a branched unsaturated fatty acid unit chain attached at either C-1 or C-3.[8,9] Compound **2** is a new eremophilane-type sesquiterpene with a branched fatty acid attached to the C-1 position, for which we propose the name xylarenone C.

Xylarenone D (**3**) was also isolated as an optically active, colorless oil ($[\alpha]^{28}_{D}$ +41). HRESIMS analysis of the quasi-molecular $[M + Na]^+$ ion at *m*/*z* 487.2666 indicated the formula $C_{26}H_{40}NaO_7$, with two additional oxygen atoms relative to compound **2**. Comparison of the ^{1}H and ^{13}C NMR spectra of **3** and **2** indicated that the only difference is that **3** has two additional OH groups, attached to C-2′ and C-11′. The methylene H_2-11′ showed HMBC correlations to C-1′ and C-2′, and the methylene H_2-3′ showed HMBC correlations to C-2′, C-4′, C-10′, and C-11′. Analysis of the remaining signals and correlations in the ^{1}H, ^{13}C, ^{1}H−^{1}H COSY, HMBC, and NOEdiff spectra confirmed that **3**, named xylarenone D, had the same overall structure as **2**, with additional hydroxyl groups placed at C-2′ and C-11′.

The optically active oil xylarenone E (**4**, $[\alpha]^{28}_{D}$ +14) displayed a quasi-molecular ion $[M - H]^-$ at *m*/*z* 479.2647, corresponding to the formula $C_{26}H_{39}O_8$. Analysis of the ^{1}H and ^{13}C NMR spectra indicated that **4** and **3** were closely related, except for the presence of a carbinolic carbon resonating at δ_C 71.7 connected to the H-7′ oxymethine group at δ 3.52 (dq, 5.5, 6.5 Hz). **4** was thus established as the 7′-hydroxyl derivative of **3** and named xylarenone E.

Eremophilane sesquiterpenes are well-known secondary metabolites of both fungi and higher plants, and members of this class display phytotoxic,[12] mycotoxic,[12] phytohormonic,[12] carcinostatic,[8] antifouling,[8] plant growth regulatory,[8] enzyme inhibitory,[13] antibacterial,[14] and cytotoxic activities.[14]

The protease inhibitory activity of compounds **1**−**4** was evaluated *in vitro* using the enzymes subtilisin and pepsin.[15] Compound **2** showed potent inhibitory activity in the pepsin (IC_{50} = 0.288 μM) and subtilisin (IC_{50} = 0.462 μM) protease assays. Compound **1** also significantly inhibited pepsin activity (IC_{50} = 2.43 μM). Substances **1**, **3**, and **4** did not display inhibitory activity on subtilisin (<10%) at any of the four concentrations tested (1.00, 0.1, 0.01, and 0.001 μM); therefore IC_{50} values could not be determined. These results suggest a negative correlation between protease inhibitory activity and hydroxyl groups on the aliphatic side chain of compounds **3** and **4**.

Data derived from sequencing and BLAST analysis of the 28S rDNA showed that isolate AM-02 had 95% sequence identity with several species within the genus *Camarops* (Ascomycota: Boliniales, Boliniaceae). Ecologically, *Camarops* spp. are considered wood-inhabiting fungi and are found in both temperate and tropical regions.[16−18] Cultures of AM-02 form abundant white aerial mycelium with the reverse side of the colony becoming orange-brown with age. *Camarops* spp. are characterized by stromatic ascomata with brown ascospores.

However, after cultivation in malt-extract agar, potato-dextrose agar, and oatmeal agar, isolate AM-02 failed to produce ascomata and ascospores. Moreover, microscopic examination revealed thin-walled hyphae, but no asexual reproductive structures.

Overall, data derived from molecular and phylogenetic analyses suggest that strain AM-02 is related to *Camarops* species and likely represents a new taxon. The production of new sesquiterpenes by this isolate supports this hypothesis.

The present report describes the new xylarenone sesquiterpenes **2**−**4**, which are xylarenones bearing polypropionate (or poly-AdoMet)-derived acids attached at C-1. These compounds are closely related to berkleasmins very recently isolated from the saprobic fungus *Berkleasmium nigroapicale* BCC 8220.[19] These unusual esters indicate that the *Camarops*-like endophyte AM-02 produces an esterification enzyme for the biosynthesis of xylarenone esters that is different from that of *Xylaria* sp. NCY2,[7] but similar to that of *B. nigroapicale* BCC 8220.[20]

EXPERIMENTAL SECTION

General Experimental Procedures. ^{1}H NMR (500 MHz), ^{13}C NMR (126 MHz), HMBC, HMQC, and COSY experiments were recorded on a Varian DRX-500 spectrometer, using the residual non-deuterated signal as internal standard. Mass spectra were measured on a Q-TOF Micromass spectrometer, using the ESI mode and MeOH−H_2O (1:1) as solvent (cone voltage 25 V). IR spectra were obtained using a Perkin-Elmer FTIR-1600 series spectrometer, using KBr pellets. Optical rotations were measured using a Perkin-Elmer polarimeter with a sodium lamp operating at 28 °C, with sample cell volume of 1 mL (MeOH). TLC analyses were performed using Merck silica gel 60 (230 mesh) and precoated silica gel 60 PF_{254}. Spots on TLC plates were visualized under UV light and by spraying with anisaldehyde−H_2SO_4 reagent followed by heating at 120 °C. Preparative HPLC was performed on a Varian Prep-Star 400 system using a Phenomenex C_{18} (250 mm × 21.2 mm) preparative column. Analytical HPLC was performed on a Varian Pro Star 230 using a Phenomenex C_{18} column (250 mm × 4.6 mm). Column chromatography (CC) was performed over reversed-phase silica gel 230−400 mesh (Merck). Fluorescence bioassay data were collected with a SynergyTM HT Multidetection microplate reader (Bio-Tek Instruments, Inc., Winooski, VT, USA), with 360 nm excitation and 460 nm emission filters, and analyzed using KC4 software (Bio-Tek Instruments) and Microsoft Windows XP. IC_{50} values were calculated using GraphPad Prism 4 software with nonlinear regression fit analysis.

Plant Material. Authenticated *Alibertia macrophylla* K. Schum. (Rubiaceae) was collected in Estação Ecológica Experimental de Mogi-Guaçu, Fazenda Campininha, Mogi-Guaçu, São Paulo, Brazil, in November 2003. Identification of *A. macrophylla* was secured by Dr. Inês Cordeiro (Institute of Botany, São Paulo, Brazil), and a voucher specimen was deposited at the Herbarium of the Institute of Botany of São Paulo, Brazil (voucher no. SP 370915).

Fungus Isolation and Identification. The endophytic fungus AM-02 was isolated from adult and healthy leaves of *A. macrophylla* that were selected and subjected to surface sterilization. Leaves were first washed with water and soap, then immersed in a 1% aqueous sodium hypochlorite solution for 5 min and in 70% aqueous EtOH for 1 min. A second washing with H_2O and soap was performed, and finally the leaves were immersed in sterile H_2O for 10 min. Sterilized leaves were cut into 2 × 2 cm pieces and deposited on a Petri dish containing PDA

(potato-dextrose agar) and anthramicine sulfate (50 μg/mL) with approximately 3 to 4 pieces on each dish.[21] The pure fungal strain was obtained after serial transfers on PDA and was deposited as AM-02 at the NuBBE fungi collection in Araraquara, Brazil (stored in sterile water at 25 °C).[21]

The endophytic fungus AM-02 was classified on the basis of a polyphasic approach (morphological, molecular, and phylogenetic analyses). The 28S rDNA (D1/D2 region) was amplified and sequenced.[22] The set of primers used for 28S rDNA amplification were NL-1 (5′- GCATATCAATAAGCG-GAGGAAAAG-3′) and NL-4 m (5′-GGTCCGTGTTTCAAGACG-3′) and for sequencing were NL-1 (5′- GCATATCAATAAGCGGAGGA-AAAG-3′), NL-2 m (5′-CTTGTGCGCTATCGGTCTC-3′), NL-3 m (5′-GAGACCGATAGCGCACAAG-3′), and NL-4 m (5′-GGTCCGTGTTT-CAAGACG-3′).

Partial 28S rDNA sequences obtained from the endophytic fungus were assembled using the Phred/phrap/Consed software. Identification was performed by comparing the contiguous rDNA sequences obtained with rDNA sequence data from reference strains available in the public CBS Fungal Biodiversity Centre database (http://www.cbs.knaw.nl/fungi/BioloMICSSequences.aspx) and GenBank (http://www.ncbi.nlm.nih.gov) by using the BLASTn routine. The sequences were aligned using the CLUSTAL X program[23] and analyzed with MEGA software version 4.0.[24] The evolutionary distances were derived from the sequence-pair dissimilarities, calculated as implemented in MEGA using the DNA substitution model.[25] The phylogenetic reconstruction was done using the neighbor-joining (NJ) algorithm,[26] with bootstrap values calculated from 1000 replicate runs using the routines included in MEGA software. The nucleotide sequence determined was deposited in the GenBank database under the accession number GQ475287. A phylogenetic tree of AM-02 is provided in the Supporting Information.

Morphological investigation was carried out in light of results obtained by the molecular analysis. Cultures grown on malt-extract agar, potato-dextrose agar, and oatmeal agar for 25 days at 28 °C were observed under a stereomicroscope (Leica MZ6, Wetzlar, Germany) in order to study the colony characteristics. In addition, details of the micromorphology were observed under a light microscope (Leica DM LS, Wetzlar, Germany) using wet mounts stained with cotton blue.

Fungal Growth. Extraction and Isolation. The endophytic fungus strain AM-02 was cultivated in nine 500 mL Erlenmeyer flasks, each containing 90 g of corn and 75 mL of H_2O. The medium was autoclaved four times (in four consecutive days) at 121 °C for 40 min. After medium sterilization, the medium was inoculated with the endophyte and incubated stationary at 25 °C for 21 days. At the end of the incubation period, the cultures were combined, ground, and extracted with MeOH (6 × 250 mL). The solvent was evaporated, to give a crude MeOH extract (5.2 g). The MeOH extract was dissolved in MeCN and defatted with hexane by liquid partitioning. The MeCN fraction was evaporated to give 0.860 g.

The crude MeCN extract was fractionated by C_{18} RPCC eluted with a H_2O−MeOH gradient (20−100% MeOH), affording 10 fractions (A−J). Fraction F (55.5 mg) was submitted to a preparative HPLC separation using H_2O−MeOH (75:25 v/v, 10 mL min, λ_{max} = 366 nm) as eluent. The new eremophilane sesquiterpene **4** was obtained (5.9 mg, t_R = 50 min). Fraction G (360 mg) was submitted to C_{18} RPCC using a gradient of H_2O in MeOH, to yield 11 subfractions (GA−GK). Subfraction GD (7.0 mg) was isolated as crystals and was identified as compound **1**. Fraction H (205 mg) was submitted to silica gel CC eluted with a gradient of EtOAc in hexane, to give 14 subfractions (HA−HN). Subfraction HA (7.4 mg) was identified as compound **2**. Subfractions HD−HH were submitted to a second silica gel CC with a stepwise gradient of EtOAc in hexane, then a gradient of MeOH in EtOAc. Eleven fractions were obtained. Subfraction HD-HH-14-20 (65 mg) was purified by silica gel CC with a gradient of MeOH in $CHCl_3$ and yielded 10 subfractions. Subfraction HD-HH-14-20-33 was identified as compound **3** (3.0 mg).

Table 2. ^{13}C NMR Data for Xylarenones C−E (2−4) ($CDCl_3$, 125 MHz)

	2	3	4
position	δ_C (mult.)	δ_C (mult.)	δ_C (mult.)
1	74.0, CH	76.6, CH	76.5, CH
2	31.0, CH_2	31.0, CH_2	31.0, CH_2
3	25.0, CH_2	25.0, CH_2	25.0, CH_2
4	37.2, CH	37.1, CH	37.2, CH
5	40.9, C	40.8, C	40.9, C
6	69.1, CH	69.1, CH	69.2, CH
7	63.2, C	63.1, C	63.7, C
8	195.2, C=O	194.4, C=O	194.4, C=O
9	125.7, CH	126.4, CH	126.4, CH
10	157.8, C	156.4, C	156.4, C
11	142.6, C	142.3, C	142.6, C
12	116.4, CH_2	116.1, CH_2	115.9, CH_2
13	63.7, CH_2	63.7, CH_2	63.7, CH_2
14	18.6, CH_3	18.3, CH_3	18.4, CH_3
15	15.7, CH_3	15.7, CH_3	15.8, CH_3
1′	175.0, C=O	174.6, C=O	174.0, C=O
2′	37.9, CH	79.0, C	79.0, C
3′	41.5, CH_2	41.8, CH_2	42.0, CH_2
4′	28.3, CH	26.7, CH	27.5, CH
5′	44.8, CH_2	45.8, CH_2	41.3, CH_2
6′	31.4, CH	31.5, CH	37.5, CH
7′	29.0, CH_2	28.7, CH_2	71.7, CH
8′	11.1, CH_3	11.1, CH_3	19.3, CH_3
9′	19.6, CH_3	18.3, CH_3	15.7, CH_3
10′	19.6, CH_3	21.6, CH_3	22.0, CH_3
11′	18.1, CH_3	68.3, CH_2	68.3, CH_2

Protease Inhibitory Activity. Pepsin from porcine gastric mucosa, recombinant type VIII subtilisin Carlsberg, Arg-Glu-(EDANS)-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Lys-(DALBCYL)-Arg fluorogenic substrate, and DMSO spectrophometric grade (Sigma-Aldrich, São Paulo, Brazil) were used. Compounds (1.0, 0.1, 0.01, and 0.001 μg/mL) were preincubated with subtilisin (37 nM) or pepsin (1.7 nM) for 1 h and then transferred to a black opaque microplate, and the substrate EDANS-DABCYL (2 μM) was injected. The final assay volume was 100 μL. Measurements were collected for 1 h at 1 min intervals. The mean of triplicates was calculated, and the IC_{50} values were estimated using the final fluorescence intensity. The value obtained in the standard assay (enzyme, buffer, and substrate) was used as a reference sample containing zero concentration of inhibitor. Compounds were dissolved in DMSO, and samples tested were diluted in the respective buffer of each enzyme, i.e., 0.1 M sodium phosphate (pH 7.5) for subtilisin and 0.1 M sodium acetate (pH 4.4) for pepsin. Since benzopyran derivatives are known to be classical inhibitors of aspartic and serinic protease, the coumarins warfarin and dicoumarol were used as inhibitory standards.[5]

Xylarenone C (2): colorless oil; $[\alpha]^{28}_D$ +14 (*c* 0.18 in MeOH); UV λ_{max} (MeOH)/nm 235; IR (KBr) ν_{max}/cm^{-1} 3442 (OH), 1730 (CO), 1679 (CO); ^{1}H NMR in Table 1; ^{13}C NMR in Table 2; HRESIMS *m*/*z* $[M + Na]^+$ 455.2773 (calculated for $C_{26}H_{40}NaO_5$, 455.2773).

Xylarenone D (3): colorless oil; $[\alpha]^{28}_D$ +41 (*c* 0.1 in MeOH); UV λ_{max} (MeOH)/nm 234; IR (KBr) ν_{max}/cm^{-1} 3450 (OH), 1733 (CO),

Table 3. Inhibitory Activity of Compounds 1−5 (IC_{50} in μM)

compound	subtilisin	pepsin
1	nd[a]	2.43
2	0.462	0.288
3	nd	115
4	nd	127.2
5	nd	nd
Warfarin	24.59	0.00832
dicoumarol	1.30	4.57

[a] nd: not determined.

1676 (CO); ^{1}H NMR in Table 1; ^{13}C NMR in Table 2; HRESIMS m/z $[M + Na]^+$ 487.2666 (calculated for $C_{26}H_{40}NaO_7$, 487.2671).

Xylarenone E (4): colorless oil; $[\alpha]^{28}_D$ +14 (c 0.11 in MeOH); UV λ_{max} (MeOH)/nm 234; IR (KBr) ν_{max}/cm^{-1} 3448 (OH), 1730 (CO), 1674 (CO); ^{1}H NMR in Table 1; ^{13}C NMR in Table 2; HRESIMS m/z $[M - H]^-$ 479.2647 (calculated for $C_{26}H_{39}O_8$, 479.2645).

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H and ^{13}C NMR spectra of compounds **1**−**4** and phylogenetic tree of endophyte AM-02. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Rodriguez, R.; Redman, R. *J. Exp. Bot.* **2008**, *59*, 1109–1114.
(2) Smith, S.; Tank, D.; Boulanger, L.; Bascom-Slack, C.; Eisenman, K.; Kingery, D.; Babbs, B.; Fenn, K.; Greene, J.; Hann, B.; Keehner, J.; Kelley-Swift, E.; Kembaiyan, V.; Lee, S.; Li, P.; Light, D.; CongMa, E.; Moore, E.; Schorn, M.; Vekhter, D.; Nunez, P.; Strobel, G.; Donoghue, M.; Strobel, S. *PLoS ONE* **2008**, *3*, e3052.
(3) Jiao, R. H.; Xu, S.; Liu, J. Y.; Ge, H. M.; Xu, H. C.; Zhu, H. L.; Tan, R. X. *Org. Lett.* **2006**, *8*, 5709–5712.
(4) Gunatilaka, A. A. L. *J. Nat. Prod.* **2006**, *69*, 509–526.
(5) Tomasselli, A. G.; Heinrikson, R. L. *Biochim. Biophys. Acta* **2000**, *1477*, 189–214.
(6) Oliveira, C. M.; Silva, G. H.; Regasini, L. O.; Zanardi, L. M.; Evangelista, A. H.; Young, M. C. M.; Bolzani, V. S.; Araújo, A. R. *Z. Naturforsch. C* **2009**, *64*, 824–830.
(7) Zhi-Yu, H.; Yao-Yao, L.; Yao-Jian, H.; Wen-Jin, S.; Yue-Mao, S. *Helv. Chim. Acta* **2008**, *91*, 46–52.
(8) McDonald, L. A.; Barbiere, L. R.; Berman, V. S.; Janso, J.; Lassota, P.; Carter, G. T. *J. Nat. Prod.* **2004**, *67*, 1565–1567.
(9) Nunes, F. M.; Oliveira, M. C. F.; Arriaga, A. M. C.; Lemos, T. L. G.; Andrade-Neto, M.; Mattos, M. C.; Mafezoli, J.; Viana, F. M. P.; Ferreira, V. M.; Rodrigues-Filho, E.; Ferreira, A. G. *J. Braz. Chem. Soc.* **2008**, *19*, 478–482.
(10) Arciniegas, A.; Pérez-Castorena, A.-L.; Reyes, S.; Contreras, J. L.; Vivar, A. R. *J. Nat. Prod.* **2003**, *66*, 225–229.
(11) Kim, S.-K.; Hatori, M.; Ojika, M.; Sakagami, Y.; Marumo, S. *Bioorg. Med. Chem.* **1998**, *6*, 1975–1982.
(12) Huang, Y. F.; Qiao, L.; Li, Lv, A.; Pei, Y. H.; Tian, L. *Chin. Chem. Lett.* **2008**, *19*, 562–564.
(13) Davis, D. A.; Brown, C. A.; Singer, K. E.; Wang, V.; Kaufman, J.; Stahl, S. J.; Wingfield, P.; Maeda, K.; Harada, S.; Yoshimura, K.; Kosalaraksa, P.; Mitsuya, H.; Yarchoan, R. *Antiv. Res.* **2006**, *72*, 89–99.
(14) Ji-Qing, X.; Li-Hong, H. *Helv. Chim. Acta* **2009**, *92*, 357–361.
(15) Babine, R. E.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1359–1472.
(16) Huhndorf, S. M.; Miller, A. N. *North Am. Fungi* **2008**, *3*, 231–239.
(17) Nannfeldt, J. A. *Svensk Bot. Tidskr.* **1972**, *66*, 335−376.
(18) Vasilyeva, L. N.; Stephenson, S. L.; Miller, A. N. *Fungal Diversity* **2007**, *25*, 219–231.
(19) González, M. C.; Anaya, A. L.; Glenn, A. E.; Saucedo-García, A.; Macías-Rubalcava, M. L.; Hanlin, R. T. *Mycotaxon* **2007**, *101*, 251–260.
(20) Isaka, M.; Srisanoh, U.; Veeranondha, S.; Choowong, W.; Lumyong, S. *Tetrahedron* **2009**, *65*, 8808–8815.
(21) Silva, G. H.; Teles, H. L.; Zanardi, L. M.; Young, M. C. M.; Eberlin, M. N.; Haddad, R.; Pfenning, L. H.; Costa-Neto, C.; Castro-Gamboa, I.; Bolzani, V. S.; Araujo, A. R. *Phytochemistry* **2006**, *67*, 1964–1969.
(22) Sette, L. D.; Passarini, M. R. Z.; Delarmelina, C.; Salati, F.; Duarte, M. C. T. *World J. Microbiol. Biotechnol.* **2006**, *22*, 1185–1195.
(23) Thompson, J. D.; Higgins, D. G.; Gibson, T. J.; Clustal, W. *Nucleic Acids Res.* **1994**, *22*, 4673–4680.
(24) Tamura, K.; Dudley, J.; Nei, M.; Kumar, S. *Mol. Biol. Evol.* **2007**, *24*, 1596–1599.
(25) Kimura, M. J. *J. Mol. Evol.* **1980**, *16*, 111–120.
(26) Saitou, N.; Nei, M. *Mol. Biol. Evol.* **1987**, *4*, 406–425.