Cytotoxic and Other Metabolites of *Aspergillus* Inhabiting the Rhizosphere of Sonoran Desert Plants[1]

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In a study to discover potential anticancer agents from rhizosphere fungi of Sonoran desert plants cytotoxic EtOAc extracts of four *Aspergillus* strains have been investigated. Two new metabolites, terrequinone A (**1**) and terrefuranone (**2**), along with N^{α}-acetyl aszonalemin (LL-S490β) (**3**) were isolated from *As. terreus* occurring in the rhizosphere of *Ambrosia ambrosoides*, whereas *As. terreus* inhabiting the rhizosphere of an unidentified *Brickellia* sp. afforded dehydrocurvularin (**4**), 11-methoxycurvularin (**5**), and 11-hydroxycurvularin (**6**). *As. cervinus* isolated from the rhizosphere of *Anicasanthus thurberi* contained two new compounds, 4*R**,5*S**-dihydroxy-3-methoxy-5-methylcyclohex-2-enone (**7**) and 6-methoxy-5(6)-dihydropenicillic acid (**8**), in addition to penicillic acid (**9**). Penicillic acid was also isolated from *As. wentii* occurring in the rhizosphere of *Larrea tridentata*. The structures of **1**−**9** were elucidated by spectroscopic methods and chemical derivatizations. Acetylation of **2** afforded 14-acetylterrefuranone (**13**) and 14-deoxy-13(14)-dehydroterrefuranone (**14**). Metabolites **1**−**9**, the dienone **14**, and 5(6)-dihydropenicillic acid (**16**) were evaluated for cytotoxicity in a panel of four human cancer cell lines and in normal human primary fibroblast cells. Compounds **4** and **5** displayed considerable cytotoxicity, whereas **1**, **6**, **9**, and **14** were found to be moderately active, with **6** and **9** exhibiting selective cytotoxicity against cancer cell lines compared with the normal fibroblast cells.

The genus *Aspergillus* (Moniliaceae), which contains about 180 recognized species,[2] has proved to be a rich source of bioactive metabolites, and even after investigations spanning over two decades, the genus still continues to provide metabolites with novel structures and interesting biological activities.[3] We have recently reported that *As. terreus* from the rhizosphere of the staghorn cholla *(Opuntia versicolor* Engelm; Cactaceae) contained a novel cyclopentadione, asterredione, the cytotoxic sesquiterpenes (+)-5,6-dihydro-6-methoxyterrecyclic acid A, (+)-5,6-dihydro-6-hydroxyterrecyclic acid A, (+)-terrecyclic acid A, and (−)-quadrone, and the cytotoxic quinones betulinan A, asterriquinone C-1, and asterriquinone D.[4] In continuation of our studies to harvest bioactive secondary metabolites from the rhizosphere of floristically diverse plant communities in the Sonoran desert,[1,4,5] we have investigated *As. terreus* Thom. occurring in the rhizosphere of the canyon ragweed [*Ambrosia ambrosioides* (Cav.) Payne; Asteraceae] and in the rhizosphere of an unidentified *Brickellia* sp. (Asteraceae), *As. cervinus* Massee, inhabiting the rhizosphere of the desert honeysuckle [*Anisacanthus thurberi* (Torr.) Gray; Acanthaceae], and *As. wentii* Wehmer occurring in the rhizosphere of the creosote bush [*Larrea tridentata* (DC.) Coville; Zygophyllaceae]. Using a bioassay-guided approach, we now report that the cytotoxic EtOAc extracts of these strains of *Aspergillus* contain four new metabolites, **1**, **2**, **7**, and **8**. Also isolated were a rare fungal metabolite, **3**,[6] and the known compounds **4**,[7,8] **5**,[8,9] **6**,[8,9] and **9**.[10] All known compounds were identified by direct comparison with authentic samples and/or by comparison of their spectroscopic data with those reported in the literature. Treatment of terrefuranone (**2**) with Ac_2O/pyridine afforded its acetate **13** and the dienone **14**. To determine the requirement of the 5(6)-unsaturation for the cytotoxicity of penicillic acid, **9** was subjected to catalytic hydrogenation, yielding 5(6)-dihydropenicillic acid (**16**).[11]

A previous study of the soil-borne *As. cervinus* has resulted in the isolation of terremutin and 3,6-dihydroxy-2,5-toluquinone,[12] whereas *As. wentii* has been reported to contain ochratoxin,[13a] substituted benzophenones and xanthones,[13b] bianthrones and secoanthraquinones,[13c] a naphthopyran, benzopyranones and maleic anhydrides,[13d] long-chain derivatives of citraconic anhydride,[13e] emodin,[13f] and aflatoxin.[13g] Herein we report the isolation of **1**−**9**, structure elucidation of the new metabolites **1**, **2**, **7**, and **8**, and cytotoxicities of **1**−**9**, **14**, and **16** toward a panel of four human cancer cell lines [NCI-H460 (non-small cell lung), MCF-7 (breast), SF-268 (CNS glioma), MIA Pa Ca-2 (pancreatic)] and normal human primary fibroblast cells (WI-38).

Results and Discussion

The cytotoxic EtOAc extract of *As. terreus*, collected from the rhizosphere of the canyon ragweed, on fractionation involving solvent−solvent partitioning followed by Sephadex LH-20 gel filtration and silica gel chromatography afforded **1**−**3**. Terrequinone A (**1**) was obtained as a purple solid that analyzed for $C_{32}H_{30}N_2O_3$ by a combination of HRFABMS and ^{13}C NMR spectroscopy. Its IR spectrum had absorption bands at 3410 and 1636 cm^{-1}, suggesting the presence of NH/OH and quinone carbonyl groups. The ^{1}H and ^{13}C NMR spectra of **1** (Table 1) had some similarities to those of isoasterriquinone[14] and suggested it to be an asterriquinone with similar substituents but located at different positions. NMR data also indicated that in **1** the central quinone moiety is monohydroxylated compared

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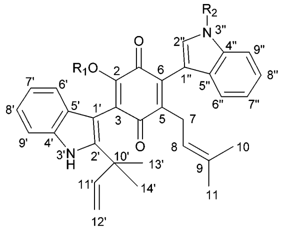
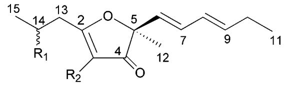


1 $R_1 = R_2 = H$

10 $R_1 = CH_3$, $R_2 = H$

11 $R_1 = Ac$, $R_2 = H$

12 $R_1 = R_2 = Ac$

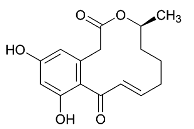


2 $R_1 = OH$, $R_2 = H$

13 $R_1 = OAc$, $R_2 = H$

14 $R_1 = H$, 13(14)-dehydro

15 $R_1 = OH$, $R_2 = CO_2H$



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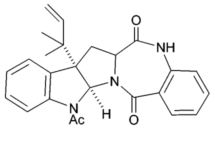

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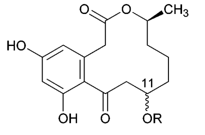
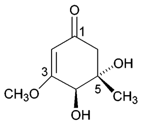


5 R = CH_3

6 R = H



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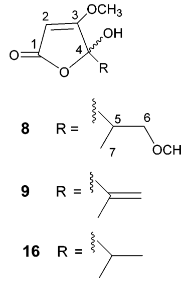

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9

16

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR Data for Terrequinone (**1**) in DMSO-d_6

position	δ_H[a]	δ_C[b]	position	δ_H[a]	δ_C[b]
1		184.4 s	8′	7.03 dt (7.0, 1.0)	121.8 d
2		153.8 s	9′	7.32 d (8.0)	111.5 d
3		118.0 s	10′		40.0 s
4		188.3 s	11′	6.16 dd (17.5, 10.5)	146.8 d
5		147.1 s	12′	5.10 br d (17.5)	111.6 t
6		135.2 s		4.99 br d (10.5)	
7	3.35 dd (12.5, 6.0)	28.8 t	13′	1.52 s	27.3 q[c]
	3.27 dd (12.5, 6.0)		14′	1.52 s	27.9 q[c]
8	5.05 m	122.5 d	1″		108.3 s
9		133.4 s	2″	7.48 d (2.0)	127.6 d
10	1.29 s	17.8 q	3″-NH	10.67 br s	
11	1.57 s	25.8 q	4″		137.3 s
1′		102.7 s	5″		128.0 s
2′		143.4 s	6″	7.39 d (8.0)	120.8 d
3′-NH	10.04 br s		7″	7.10 dt (8.0, 1.0)	120.5 d
4′		136.5 s	8″	7.19 dt (8.0, 1.0)	122.6 d
5′		129.9 s	9″	7.49 d (8.0)	112.6 d
6′	7.21 d (7.5)	119.7 d	2-OH	8.87 br s	
7′	6.93 dt (8.0, 1.0)	119.6 d			

[a] Multiplicites deduced from HSQC; coupling constants (*J* values in Hz) are in parentheses. Assignments are based on HSQC and HMBC spectra. [b] Multiplicites deduced from DEPT. Assignments are based on HSQC and HMBC spectra. [c] Signals may be interchanged.

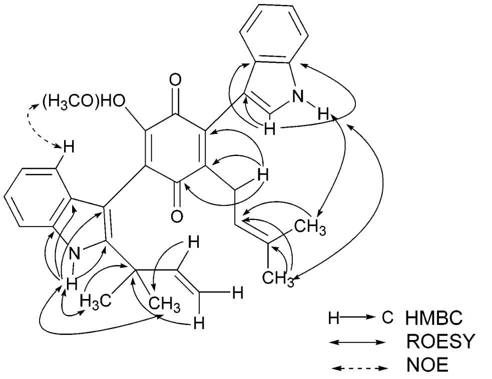

Figure 1. HMBC and ROESY correlations for **1** and NOE correlations for **10**.

with all known asterriquinones, which contain dioxygenated quinone moieties. This was further supported by the presence of a 1H broad singlet at δ 8.87, which disappeared on methylation, in addition to two 1H broad singlets at δ 10.67 and 10.04 due to indole NH groups. The ^1H NMR spectrum of terrequinone A also suggested the presence of two indole residues, one of which is 3-substituted and the other 2,3-disubstituted, two 3H singlets at δ 1.29 and 1.57, and a 6H singlet at δ 1.52 in addition to two independent spin systems. On the basis of their chemical shifts, these 3H and 6H singlets were assigned to four CH_3 groups on quaternary aliphatic/olefinic carbons. The $^1H-^1H$ COSY spectrum of **1** indicated that one of the spin systems consisted of two 1H double doublets at δ 3.35 and 3.27 (J = 12.5 and 6.0 Hz) and a 1H multiplet at δ 5.05. In the HMBC spectrum of **1** (Figure 1) the protons at δ 3.35 and 3.27 showed strong correlations with carbons at δ 122.5 (to which the proton at δ 5.05 is attached as determined by the HSQC spectrum), 188.3 (quinone carbonyl), 147.1 (C-5 of quinone), and 133.4 (olefinic quaternary carbon). The strong HMBC correlations observed between the 3H singlets at δ 1.57 and 1.29 with the olefinic carbon signals at δ 133.4 (C-9) and 122.5 (C-8) together with the above observations confirmed the presence of a dimethylallyl group attached to C-5 of the quinone moiety of **1**. The remaining spin system of terrequinone A consisted of two 1H doublets [δ 5.10 (J = 17.5 Hz) and 4.99 (J = 10.5 Hz)] and a 1H double doublet [δ 6.16 (J = 17.5 and 10.5 Hz)], suggesting the presence of a vinyl group on a quaternary carbon. In the HMBC spectrum, the protons at δ 5.10 and 4.99 showed strong correlations with the quaternary carbon signal at δ 40.0 (C-10′), which also had cross-peaks with the 6H singlet at δ 1.52, and the olefinic signal at δ_H 6.16 (H-11′) with the CH_3 signals at δ_C 27.9 (C-13′/C-14′) and 27.3 (C-13′/C-14′). The foregoing suggested the presence of a 1,1-dimethyprop-2-enyl (inverted γ,γ-dimethylallyl) group in **1** at C-2′ of the 2,3-disubstituted indole residue (see above). This was confirmed by the presence of a strong ROESY correlation between the 6H singlet at δ 1.52 and the NH signal at δ_H 10.04 of this indole ring, and the resistance of this NH to undergo acetylation on treatment of **1** with Ac_2O/pyridine at room temperature. The remaining NH signal of **1** at δ_H 10.67, which underwent ready acetylation under the above conditions, probably due to the absence of steric crowding at C-2″ of this indole ring, showed strong ROESY correlations with CH_3 signals at δ 1.57 and 1.29, suggesting the 3-substituted indole residue and the dimethylallyl group to be on the same side of the central quinone core of terrequinone A (**1**). Methylation of **1** with CH_3I/K_2CO_3 in acetone at room temperature afforded the monomethyl derivative **10**. In the NOE spectrum of **10**, irradiation of the signal due to 6′-H (at δ_H 7.21) of the disubstituted indole moiety induced an enhancement of the 3H singlet at δ 3.72 of the newly introduced Me group, indicating that the OH group and the 2,3-disubstituted indole residue of **1** are on the same side of the quinone core. Acetylation of **1** afforded two products, which were identified as its monoacetate (**11**) and the diacetate (**12**). The ^1H NMR spectrum of **12** indicated that, in addition to the OH of the quinone system, the NH of the monosubstituted indole ring has undergone acetylation as evident from the disappearance of the signal at δ_H 10.67 and significant downfield shifts of 2″-H and 9″-H of **12** compared with those of **1**. On the basis of the foregoing evidence the structure of terrequinone A was elucidated as 2-hydroxy-3-[2′-(1,1-dimethylprop-2-en)indol-3′-yl)]-5-(3-methylbut-2-enyl)-6-(indol-3′-yl)cyclohex-2,5-diene-1,4-dione (**1**).

The molecular formula of terrefuranone (**2**) was determined as $C_{14}H_{20}O_3$ from its HRFABMS and ^{13}C NMR data and indicated five degrees of unsaturation. The IR absorption bands at 3425 and 1697 cm^{-1} and the ^{13}C NMR signals at δ 65.6 and 204.7 suggested the presence of OH and α,β-unsaturated ketone carbonyl groups. The ^1H NMR spectrum of **2** consisted of one 3H singlet at δ 1.47 assigned to a CH_3 group on a quaternary carbon (C-5), one olefinic singlet at δ 5.43, and two independent spin systems. The $^1H-^1H$ COSY spectrum of **2** indicated that one of the spin systems consisted of a 3H doublet ($J = 6.5$ Hz) at δ 0.97 attributable to a CH_3 attached to an oxygenated methine at δ 4.21 (1H, m), which in turn is attached to a methylene group at δ 2.69 (2H, m). The chemical shift of this CH_2 indicated that it is attached to an olefinic carbon, suggesting the presence of the fragment $CH_3CH(OH)CH_2C{=}C$ in **2**. This was further confirmed by the treatment of **2** with Ac_2O/pyridine, which resulted in the formation of its acetate **13** and the corresponding dienone, 13-deoxy-12(13)-dehydroterrefuranone (**14**). The ^1H NMR spectrum of **13** showed downfield shifts of all the protons of this fragment, especially the shift from δ 4.21 to 5.25 of the signal assigned to the proton geminal to the OH group. The $^1H-^1H$ COSY spectrum indicated that the remaining spin system of **2** consisted of a 3H triplet ($J = 6.5$ Hz) at δ 0.97 attributable to a CH_3 attached to a methylene at δ 2.08 (m) and that the latter is attached to a methine group at δ 5.78 (m), which is a part of a conjugated diene [δ 5.96 (dd, $J = 15.0$ and 9.5 Hz), 6.26 (dd, $J = 15.5$ and 4.5 Hz), 5.52 (d, $J = 15.5$ Hz)] attached to a quaternary carbon. The large coupling constants of the olefinic protons of this spin system together with these ^1H NMR data identified this fragment as *E*,*E*-hexa-1,3-diene. These two spin systems and the CH_3 group on C-5 (see above) accounted for $C_{10}H_{19}O$ and 2 units of unsaturation. Thus, the remaining fragment of **2** has the partial formula C_4HO_2 with 3 units of unsaturation and a carbonyl function, which suggested this to be a trisubstituted dihydrofuran-4-one. The chemical shift (δ_H 5.43) of the olefinic singlet and the ready dehydration of **2** on treatment with Ac_2O/pyridine yielding the corresponding dienone **14** indicated the attachment of the $CH_3CH(OH)$-CH_2 fragment (see above) to C-2 of the dihydrofuran-4-one. HSQC and HMBC data further confirmed the presence of these three fragments, and HMBC correlations (Figure 2) were used to establish their connectivities, leading to the planar structure **2** proposed for terrefuranone. It remained to determine the stereochemical dispositions of the groups at C-5 and C-14 in **2**. The configuration at C-5 was assigned using CD data for the dienone **14** derived from **2**. The CD spectrum of **14** consisted of a curve (R band) with a strong negative first Cotton effect (306 nm) and a weak positive second Cotton effect (275 nm), indicating negative (*S*) chirality at C-5.[15] Attempts to prepare the Mosher ester of **2** resulted in the formation of the dienone **14** with no trace of the desired ester, and this precluded the determination of the configuration at C-14. On the basis of the foregoing evidence, the structure of terrefuranone was elucidated as 5β-(1*E*,3*E*)-1,3-hexadienyl-4,5-dihydro-2-(2-hydroxypropyl)-5α-methylfuran-4-one (**2**). Although the related anti-HIV active carboxylic acid, nivefuranone C (**15**), with undefined configuration at C-5 and C-14, is known to occur in *As. niveus*,[16] its spectroscopic data have not been reported in the literature.

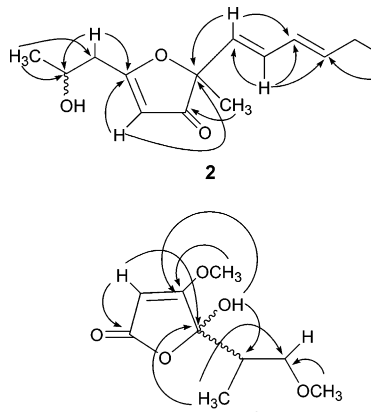

Figure 2. Selected HMBC correlations for **2** and **8**.

Bioassay-guided fractionation of the cytotoxic EtOAc extract of *As. terreus*, isolated from the rhizosphere of an unidentified *Brickellia* sp., involving solvent−solvent partition, followed by Sephadex LH-20 gel filtration and repeated silica gel and reversed-phase chromatography furnished three cytotoxic compounds, **4**−**6**. These were identified as dehydrocurvularin (**4**), 11-methoxycurvularin (**5**), and 11-hydroxycurvularin (**6**) by comparison of their spectroscopic data with those reported in the literature[7,9] and by direct comparison with authentic samples.[8] Although **5** and **6** have been reported as metabolites of several fungi,[7−9] isolation of each of these as a mixture of 11α- and 11β-epimers suggests their possible artifactual origin, probably by a Michael-type addition of MeOH and H_2O to the enone system of dehydrocurvularin (**4**) during the processing of these microorganisms.

The EtOAc extract of *As. cervinus*, isolated from the rhizosphere of *A. thurberi*, was selected for detailed investigation on the basis of its cytotoxicity and because of the absence of any reported biological activity of the metabolites previously encountered in this fungal species.[12] Fractionation of the EtOAc extract as above furnished **7**−**9**. The molecular formula of compound **7**, isolated as an optically active colorless semisolid, was deduced as $C_8H_{12}O_4$ from its HRFABMS and ^{13}C NMR spectra and indicated three degrees of unsaturation. IR absorption bands at 3402 and 1620 cm^{-1} suggested the presence of OH and α,β-unsaturated ketone carbonyl groups. The ^1H NMR spectrum of **7** indicated the presence of two 3H singlets at δ 1.34 and 3.76 due to CH_3 and OCH_3, respectively, two 1H doublets ($J = 16.5$ Hz) at δ 2.68 and 2.50, and two 1H singlets at δ 4.32 and 5.35, accounting for all but two protons which were suspected to belong to two OH groups. The ^{13}C NMR spectrum, assigned on the basis of DEPT, HSQC, and HMBC spectra, showed the presence of a ketone carbonyl (δ 196.1), two olefinic carbons (δ 101.5 and 174.3), of which one was oxygenated (δ 174.3), two oxygenated sp^3 carbons (δ 74.5 and 73.7), a methoxy carbon (δ 56.6), and a methyl carbon (δ 22.5). The UV spectrum of **7** had an absorption band at 250.5 nm, indicating the presence of a cyclic β-oxygenated-enone structure. The chemical shifts and coupling constants of the 1H doublets at δ_H 2.68 and 2.50 suggested that they belong to a $COCH_2$ moiety of a cyclic system with its CH_2 group attached to a quaternary carbon. In the HMBC spectrum the signal at δ_H 2.68 had cross-peaks with signals at δ_C 196.1, 101.5, and 73.7, and the signal at δ_H 4.32 showed correlations with those at δ_C 174.3

and 73.7. These HMBC data suggested that the CH_2 of the $COCH_2$ moiety is attached to the oxygenated quaternary carbon at δ 73.7 and that the oxygenated carbon at δ 74.5 (δ_H 4.32) is attached to the oxygenated carbon of the olefin of the enone system. In the NOESY spectrum **7** had strong spatial correlations between δ_H 2.68 and 4.32 and between δ_H 2.50 and 1.34, indicating a *trans* relationship between the vicinal OH groups. On the basis of the above data, the structure of **7** was elucidated as 4*R**,5*S**-dihydroxy-3-methoxy-5-methylcyclohex-2-enone.

Compound **8** was obtained as an optically active colorless oil. Its molecular formula, $C_9H_{14}O_5$, determined by a combination of HRFABMS and ^{13}C NMR spectroscopy indicated three degrees of unsaturation. It showed IR absorption bands at 3356, 1751, and 1643 cm^{-1}, suggesting the presence of OH and α,β-unsaturated lactone groups. The UV spectrum of **8** showed very close resemblance to that of **7** with an absorption band at 249.5 nm, indicating the presence of a cyclic β-oxygenated-enone structure. Its ^1H NMR spectrum had three singlets, one due to an olefinic proton at δ 5.02 and two due to OCH_3 groups at δ 3.87 and 3.38, a D_2O exchangeable proton at δ 6.70, and signals assignable to the $-CH(CH_3)CH_2O-$ spin system. The ^{13}C NMR spectrum of **8** when analyzed with the help of HSQC showed the presence of one CH_3, two OCH_3, one CH_2 bearing an oxygen atom, an olefinic CH, and three quaternary carbons. The above data indicated **8** to be a furan-2-enone bearing $-OCH_3$, $-OH$, and $-CH(CH_3)CH_2OCH_3$ groups. The points of attachments of these groups to the furan-2-enone ring system were determined with the help of HMBC correlations (Figure 2). On the basis of the foregoing evidence the structure of **8** was elucidated as 6-methoxy-5(6)-dihydropenicillic acid. Analysis of NMR coupling constants, NOE data, and the CD spectrum did not permit definitive assignment of the configuration at C-4 and C-5 of **8**. Compound **9** was identified as penicillic acid by comparison of its 1D and 2D NMR spectral data with those reported in the literature.[10] The presence of penicillic acid (**9**) in the same extract and the use of MeOH during the extraction of the fungus suggested possible artifactual origin of **8** from **9**. However, **9** failed to react with MeOH to produce **8** under a variety of conditions including such harsh conditions as refluxing MeOH in the presence of *p*-toluenesulfonic acid (*p*-TSA), indicating that **8** is a genuine natural product. Penicillic acid (**9**) has previously been reported from several *Aspergillus* and *Penicillium* species.[10] Co-occurrence of **7** and **9** in *As. cervinus* is noteworthy, as compounds structurally related to **7** (e.g., 6-methoxy-2-methyl-1,4-benzoquinone) have been implicated as biosynthetic precursors of penicillic acid (**9**).[17] Bioactivity-guided fractionation of the cytotoxic EtOAc extract of *As. wentii*, isolated from the rhizosphere of *L. tridentata*, afforded penicillic acid (**9**) as the only cytotoxic constituent of this extract.

Compounds **1**−**9**, **14**, and **16** were evaluated for in vitro cytotoxicity against a panel of four cancer cell lines (NCI-H460, MCF-7, SF-268, and MIA Pa Ca-2) and normal human primary fibroblast cells (WI-38). Cells were treated with test compounds for 72 h in RPMI-1640 media supplemented with 10% fetal bovine serum, and cell viability was evaluated by the MTT assay.[18] The concentrations resulting in 50% inhibition of cell proliferation/survival (IC_{50}) as measured by this assay are given in Table 2. Of those tested, terrequinone A (**1**), dehydrocurvularin (**4**), 11-methoxycurvularin (**5**), 11-hydroxycurvularin (**6**), penicillic acid (**9**), and 13-deoxy-12(13)-dehydroterrefuranone (**14**) were found to be cytotoxic, with IC_{50} values ranging from 0.90 to 57.50 μM. The most cytotoxic are the curvularins **4**−**6**. It is significant that penicillic acid (**9**) is selectively cytotoxic against non-small cell lung and pancreatic cancer cell lines used in this study. Compounds **4**−**6** have been reported to inhibit sea urchin embryogenesis by acting on components of the mitotic apparatus,[19] and a recent patent application suggests their weak interaction with heat shock protein 90 (Hsp90),[20] a promising target for anticancer drug discovery.[21] Penicillic acid (**9**) has been reported to be cytotoxic to A2780 human ovarian carcinoma,[22a] Chinese hamster ovary cells,[22b] and HeLa cells[22c] and was found to inhibit the growth of sarcoma originally induced by it.[22d] The mechanism of cytotoxicity of **9** has been determined to be due to its ability to bind to SH groups in macromolecules,[22e] induction of DNA single-strand breaks,[22b] and inhibition of DNA synthesis.[22c] It has recently been demonstrated that penicillic acid (**9**) inhibits Fas ligand-induced apoptosis by targeting self-processing of caspase-8.[22f] The absence of any cytotoxic activity of its derivatives **8** and **16** suggests the requirement of the isopropenyl moiety for the cytotoxicity of **9**. Studies to elucidate the molecular mechanism(s) of action of the most potent cytotoxins **4** and **5** and animal studies to evaluate their antitumor potential are currently in progress.

Table 2. Cytotoxicities of Compounds **1**−**9**, **14**, and **16** against a Panel of Four Tumor Cell Lines and Normal Human Primary Fibroblast Cells[a]

	cell line[b]				
compound	NCI-H460	MCF-7	SF-268	MIA Pa Ca-2	WI-38
1	5.60	6.80	13.90	5.40	NT[c]
4	1.10	1.30	2.50	1.90	3.60
5	0.90	0.60	0.90	1.20	1.70
6	2.10	2.00	4.10	3.30	11.60
9	5.80	12.80	20.00	8.00	57.50
14	5.50	11.70	6.80	6.30	NT[c]
doxorubicin	0.01	0.07	0.04	0.05	0.30

[a] Results are expressed as IC_{50} values in μM; compounds **2**, **3**, **7**, **8**, and **16** were found to be inactive in all cell lines at 10.0 μg/mL. [b] Key: NCI-H460 = human non-small cell lung cancer; MCF-7 = human breast cancer; SF-268 = human CNS cancer (glioma); MIA Pa Ca-2 = human pancreatic cancer; WI-38 = normal human primary fibroblast cells. [c] NT = not tested.

Experimental Section

General Experimental Procedures. Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO Dip-370 polarimeter using $CHCl_3$ as solvent. CD spectrum was obtained using a JASCO J-805 spectropolarimeter. IR spectra for KBr disks were recorded on a Shimadzu FTIR-8300 spectrometer. 1D and 2D NMR spectra were recorded in $CDCl_3$, acetone-d_6, and DMSO-d_6 and using residual solvents as internal standards with a Bruker DRX-500 instrument at 500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR and a Bruker DRX-600 instrument at 600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR. The chemical shift values (δ) are given in parts per million (ppm), and the coupling constants are in Hz. Low-resolution and high-resolution MS were recorded respectively on Shimadzu LCMS QP8000α and JEOL HX110A spectrometers.

Cytotoxicity Bioassays. The tetrazolium-based colorimetric assay (MTT assay)[18] was used for the in vitro assay of cytotoxicity to human non-small cell lung carcinoma (NCI-H460), human breast carcinoma (MCF-7), human glioma (SF-268), human pancreatic cancer (MIA Pa Ca-2) cell lines, and normal human primary fibroblast (WI-38) cells as previously reported.[4] All samples for cytotoxicity assays were dissolved in DMSO. During bioassay-guided fractionation, cytotoxicity of fractions was monitored using the NCI-H460 cell line.

Fungal Isolations. The fungal strains were isolated from the rhizospheres of *Ambrosia ambrosoides* (collected from Tucson Mountains, AZ; herbarium sample accession No. AH-00-21), *Brickellia* sp. (from Sycamore Springs in Greasewood Mountains, AZ; accession No. AH-02-30), *Anicasanthus thurberi* (from Santa Rita Mountains near Sonoita, AZ; accession No. AH-00-70), and *Larrea tridentata* (from Puerto Blanco Mountains, AZ; accession No. AH-00-115). All plant species were identified by Dr. Annita Harlan of the University of Arizona. Identification of the isolated fungal strains was made by Ms. Donna Bigelow, Ms. Jun Zhang, and Dr. Elizabeth Pierson (all of Department of Plant Sciences, University of Arizona) by the analysis of the ITS regions of the ribosomal DNA as described previously.[4] Excised roots of each plant (1 cm long sections; ca. 5 g) were separately placed in 5 mL of phosphate-buffered saline (PBS, 0.1M, pH = 7.4), and microorganisms were detached from the roots by vortexing and sonication. Serial dilutions of the resulting suspensions were placed on Petri dishes containing potato dextrose agar (PDA, Difco, Plymouth, MN) supplemented with chloramphenicol and streptomycin. After 4 days of incubation at 25 °C, single colonies from each Petri dish were transferred to water agar Petri dishes containing the same antibiotics, and after 3 days pure fungal cultures were obtained by hyphal tipping. Each fungal strain is deposited in the Division of Plant Pathology and Microbiology, Department of Plant Sciences, and Southwest Center for Natural Products Research and Commercialization of the University of Arizona microbial culture collections under the following accession numbers: *As. terreus* from *A. ambrosoides*, AH-00-21-F12; *As. terreus* from *Brickellia* sp., AH-02-30-F7; *As. cervinus* from *A. thurberi*, AH-00-70-F11; and *As. wentii* from *L. tridentata*, AH-00-115-F15. Each organism was subcultured on PDA, and for long-term storage isolates were subcultured on PDA slants, overlaid with 40% glycerol, and stored at −80 °C.

Cultivation and Isolation of Metabolites of *As. terreus* from the Rhizosphere of *A. ambrosoides*. For isolation of secondary metabolites, the fungus was cultured in 40 T-flasks (800 mL), each containing 135 mL of PDA coated on five sides of the flasks, maximizing the surface area for fungal growth (total surface area/flask ca. 460 cm^2). After incubation for 28 days at 27 °C, MeOH (200 mL/T-flask) was added to all 40 T-flasks, which were sonicated and shaken in a rotary shaker for 12 h at room temperature, and the resulting extract was filtered through Whatman No. 1 filter paper and a layer of Celite 545. The filtrate was concentrated to one-fourth of its original volume and extracted with EtOAc (5 × 300 mL). Combined EtOAc extracts were evaporated under reduced pressure to afford a brown semisolid (657.4 mg), a portion (477.0 mg) of which was partitioned between hexane and 80% aqueous MeOH. The cytotoxic 80% aqueous MeOH fraction was diluted to 50% aqueous MeOH with H_2O and extracted with $CHCl_3$. Evaporation of $CHCl_3$ under reduced pressure yielded a brown semisolid (184.0 mg), which was subjected to gel permeation chromatography on a column of Sephadex LH-20 (2.0 g) in hexane/CH_2Cl_2 (1:4) and eluted with hexane/CH_2Cl_2 (1:4) (50 mL), CH_2Cl_2/acetone (3:2) (50 mL), and CH_2Cl_2/MeOH (1:1) (50 mL) to furnish six fractions, 1−6 (25 mL each), of which fraction 2 (90.4 mg) was found to be the most cytotoxic. Column chromatography of this fraction (90.0 mg) on silica gel (1.5 g) and elution with CH_2Cl_2 (50.0 mL) and CH_2Cl_2/MeOH (50:1) (50 mL) afforded **1** (6.0 mg) and three subfractions, A−C. Chromatography of fraction A (50.9 mg) on silica gel (0.3 g) by elution with hexane/EtOAc (1.5:1) afforded **2** (5.0 mg) and **3** (30.0 mg).

Terrequinone A (1): purple powder; mp 160−165 °C; UV (MeOH) λ_{max} (log ϵ) 223.0 (5.63), 274.5 (5.18), 360.0 (4.24) nm; IR (KBr) ν_{max} 3410, 2932, 1636, 1435, 1096, 741 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; APCIMS (+)-ve mode *m/z* 491 [M + 1]$^+$; APCIMS (−)-ve mode *m/z* 489 [M − 1]$^+$; HRFABMS *m/z* 490.2260 [M]$^+$ (calcd for $C_{32}H_{30}N_2O_3$, 490.2256).

Terrefuranone (2): colorless oil; $[\alpha]^{20}{}_D$ +16.8 (*c* 0.13 MeOH); UV (MeOH) λ_{max} (log ϵ) 233 (4.34), 265 (3.94) nm; IR (KBr) ν_{max} 3425, 2924, 2855, 1697, 1589, 1373, 1111 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.26 (1H, dt, *J* = 15.5, 4.5 Hz, H-7), 5.96 (1H, dd, *J* = 15.0, 9.5 Hz, H-8), 5.78 (1H, m, H-9), 5.52 (1H, d, *J* = 15.5 Hz, H-6), 5.43 (1H, s, H-3), 4.21 (1H, m, H-14), 2.69 (2H, m, CH_2-13), 2.08 (2H, m, CH_2-10), 1.47 (3H, s, CH_3-12), 1.30 (3H, d, *J* = 6.5 Hz, CH_3-15), 0.97 (3H, t, *J* = 6.5 Hz, CH_3-11); ^{13}C NMR (125 MHz, $CDCl_3$) δ 204.7 (C, C-4), 188.6 (C, C-2), 138.8 (CH, C-9), 131.1 (CH, C-7), 127.9 (CH, C-8), 126.5 (CH, C-6), 90.2 (C, C-5), 65.6 (CH, C-14), 40.4 (CH_2, C-13), 25.7 (CH_2, C-10), 23.4 (CH_3, C-15), 22.3 (CH_3, C-12), 13.3 (CH_3, C-11); APCIMS (+)-ve mode *m/z* 237 [M + 1]$^+$; APCIMS (−)-ve mode *m/z* 235 [M − 1]$^+$; HRFABMS *m/z* 237.1490 [M + 1]$^+$ (calcd for $C_{14}H_{21}O_3$, 237.1491).

N^a-Acetylaszonalemin (3): colorless amorphous solid; ^{1}H and ^{13}C NMR and MS data were consistent with those reported in the literature.[6b]

Methylation of Terrequinone A. Methyl iodide (0.2 mL) and K_2CO_3 (10.0 mg) were added to a stirred solution of **1** (1.2 mg) in acetone (0.2 mL) at 0 °C. After 5 min at 0 °C, the ice bath was removed and the reaction mixture was stirred at 25 °C until the starting material disappeared (TLC control). It was then filtered, solvent was removed under reduced pressure, and the crude product was purified by preparative TLC (silica gel) using 1% MeOH in CH_2Cl_2 as eluant to give **10** (1.2 mg).

Terrequinone A monomethyl ether (10): dark brown solid; ^{1}H NMR (600 MHz, acetone-d_6) δ 10.68 (1H, brs, NH), 10.15 (1H, brs, NH), 7.51 (1H, d, *J* = 7.8 Hz, H-6″), 7.49 (1H, d, *J* = 2.4 Hz, H-2″), 7.38 (1H, d, *J* = 7.8 Hz, H-9″), 7.35 (1H, d, *J* = 8.0 Hz, H-6′), 7.25 (1H, d, *J* = 8.0 Hz, H-9′), 7.18 (1H, t, *J* = 7.8 Hz, H-7″), 7.09 (1H, t, *J* = 8.0 Hz, H-7′), 7.07 (1H, t, *J* = 7.8 Hz, H-8″), 6.98 (1H, t, *J* = 8.0 Hz, H-8′), 6.14 (1H, dd, *J* = 17.5, 10.6 Hz, H-11′), 5.09 (1H, dd, *J* = 17.4, 0.8 Hz, H-12′a), 5.05 (1H, m, H-8), 5.02 (1H, dd, *J* = 10.6, 0.9 Hz, H-12′b), 3.72 (3H, s, OCH_3), 3.32 (1H, dd, *J* = 12.9, 7.4 Hz, H-7a), 3.21 (1H, dd, *J* = 13.5, 6.5 Hz, H-7b), 1.55 (3H, s, CH_3), 1.51 (3H, s, CH_3), 1.50 (3H, s, CH_3), 1.29 (3H, s, CH_3); APCIMS (−)-ve mode *m/z* 503 [M − H]$^+$; HRFABMS *m/z* 504.2448 [M]$^+$ (calcd for $C_{33}H_{32}N_2O_3$, 504.2413).

Acetylation of Terrequinone A. Acetic anhydride (0.2 mL) was added to a solution of terrequinone A (**1**) (2.0 mg) in pyridine (0.1 mL) and stirred at 25 °C until the starting material disappeared (TLC control). Pyridine and excess Ac_2O were removed under reduced pressure, and the products were separated by preparative TLC (silica gel) using 1% methanol in CH_2Cl_2 as eluant to give **11** (0.9 mg) and **12** (0.9 mg).

Terrequinone A monoacetate (11): dark brown solid; ^{1}H NMR (600 MHz, acetone-d_6) δ 10.74 (1H, brs, NH), 10.30 (1H, brs, NH), 7.51 (1H, d, *J* = 2.6 Hz, H-2″), 7.52 (1H, d, *J* = 7.0 Hz, H-6″), 7.39 (1H, d, *J* = 7.0 Hz, H-9″), 7.35 (1H, d, *J* = 7.8 Hz, H-6′), 7.24 (1H, d, *J* = 7.8 Hz, H-9′), 7.19 (1H, dt, *J* = 7.0, 0.9 Hz, H-7″), 7.10 (1H, dt, *J* = 7.8, 0.9 Hz, H-7′), 7.08 (1H, dt, *J* = 7.0, 0.9 Hz, H-8″), 6.98 (1H, dt, *J* = 7.8, 0.9 Hz, H-8′), 6.16 (1H, dd, *J* = 17.4, 10.6 Hz, H-11′), 5.08 (1H, dd, *J* = 17.4, 0.8 Hz, H-12′a), 5.07 (1H, m, H-8), 5.04 (1H, dd, *J* = 10.6, 1.2 Hz, H-12′b), 3.39 (1H, dd, *J* = 13.5, 7.4 Hz, H-7a), 3.27 (1H, dd, *J* = 13.5, 6.7 Hz, H-7b), 1.96 (3H, s, OAc), 1.57 (3H, s, CH_3), 1.51 (3H, s, CH_3), 1.50 (3H, s, CH_3), 1.29 (3H, s, CH_3); APCIMS (−)-ve mode *m/z* 531 [M − H]$^+$.

Terrequinone A diacetate (12): dark brown solid; ^{1}H NMR (600 MHz, acetone-d_6) δ 10.36 (1H, brs, NH), 7.91 (1H, s, H-2″), 8.47 (1H, d, *J* = 8.3 Hz, H-6″), 7.41−7.38 (3H, m, H-7″, H-8″, H-9″), 7.35 (1H, d, *J* = 7.8 Hz, H-6′), 7.24 (1H, d, *J* = 7.8 Hz, H-9′), 7.09 (1H, dt, *J* = 7.8, 0.7 Hz, H-7′), 6.98 (1H, dt, *J* = 7.8, 0.7 Hz, H-8′), 6.17 (1H, dd, *J* = 17.4, 10.7 Hz, H-11′), 5.07 (1H, d, *J* = 17.4 Hz, H-12′a), 5.06 (1H, d, *J* = 10.6 Hz, H-12′b), 5.02 (1H, brt, H-8), 3.36 (1H, dd, *J* = 14.4, 6.9 Hz, H-7a), 3.27 (1H, dd, *J* = 14.4, 5.2 Hz, H-7b), 1.96 (3H, s, OAc), 1.55 (3H, s, CH_3), 1.51 (3H, s, CH_3), 1.50 (3H, s, CH_3), 1.29 (3H, s, CH_3); APCIMS (−)-ve mode *m/z* 573 [M − H]$^+$.

Acetylation of Terrefuranone (2). Compound **2** (1.0 mg) was acetylated with Ac_2O (0.5 mL) in pyridine (0.5 mL) with stirring at room temperature for 12 h. The reaction mixture was purified on preparative silica gel eluting with hexane/EtOAc (3:1) to yield 14-acetyl terrefuranone (**13**) (0.3 mg) and 14-deoxy-13(14)-dehydroterrefuranone (**14**) (0.6 mg). Compound **13**: colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.26 (1H, m, H-2″), 5.95 (1H, m, H-3″), 5.79 (1H, m, H-4″), 5.50 (1H, dd, *J* = 15.5, 2.5 Hz, H-1″), 5.39 (1H, s, H-4), 5.25 (1H, m, H-2′), 2.78 (2H, m, H-1′), 2.07 (2H, m, H-5″), 1.99 (3H, s, CH_3CO), 1.45 (3H, s, H-1‴), 1.32 (3H, d, *J* = 6.5 Hz, H-3′), 0.97 (3H, t, *J* = 7.5 Hz, H-6″); APCIMS (+)-ve mode *m/z* 279 $[M + 1]^+$, 320 $[M + CH_3CN]^+$, 260 $[M + H - HOAc]^+$, 260 $[M + CH_3CN - HOAc]^+$. Compound **14**: colorless oil; UV (MeOH) λ_{max} (log ϵ) 235 (3.36), 276 (3.36), 306 (3.00) nm; CD λ_{max} (4.23×10^{-4} M EtOH) nm 306 ([θ] −37770); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.83 (1H, dq, *J* = 15.5, 7.0 Hz, H-2′), 6.26 (1H, m, H-2″), 6.24 (1H, dd, *J* = 15.5, 1.5 Hz, H-1′), 5.96 (1H, dd, *J* = 15.0, 10.2 Hz, H-3″), 5.77 (1H, m, H-4″), 5.56 (1H, d, *J* = 15.2 Hz, H-1″), 5.36 (1H, s, H-4), 2.07 (2H, m, H-5″), 1.95 (3H, dd, *J* = 7.0, 1.5 Hz, H-3′), 1.48 (3H, s, H-1‴), 0.97 (3H, t, *J* = 7.3 Hz, H-6″); APCIMS (+)-ve mode *m/z* 219 $[M + 1]^+$.

Cultivation and Isolation of Metabolites of *As. terreus* from the Rhizosphere of *Brickellia* sp. For isolation of secondary metabolites, the fungus was cultured on PDA in 60 T-flasks for 30 days at 27 °C. Extraction and liquid−liquid fractionation as for *As. terreus* from *A. ambrosoides* (see above) afforded the cytotoxic $CHCl_3$ fraction as a dark brown solid (486.1 mg). A portion (300.0 mg) of this was subjected to gel permeation chromatography on a column of Sephadex LH-20 (10.0 g) in hexane/CH_2Cl_2 (1:4) and eluted with hexane/CH_2Cl_2 (1:4) (75 mL), CH_2Cl_2/acetone (3:2) (100 mL), and CH_2Cl_2/acetone (1:4) (50 mL). Seventeen fractions (ca. 15 mL each) were collected and combined on the basis of their TLC patterns to yield three cytotoxic fractions, A−C. Column chromatography of fraction A (91.5 mg) on reversed-phase silica gel (4.0 g) and elution with $MeCN/H_2O$ (32:68) afforded a cytotoxic subfraction (40.9 mg), a portion (17.0 mg) of which was further purified by preparative TLC on silica gel (CH_2Cl_2/2-PrOH, 95:5) to afford **4** (4.6 mg). Fraction B (118.0 mg) was subfractionated on silica gel (5.0 g) using a gradient of EtOAc in hexane to afford a cytotoxic subfraction (62.4 mg). Further purification of this by column chromatography on reversed-phase silica gel (3.0 g) and elution with increasing amounts of MeCN in H_2O yielded a crude fraction (11.0 mg), which on further purification by preparative TLC on silica gel (CH_2Cl_2/2-PrOH, 95:5) afforded pure **5** (9.0 mg). Evaporation of the cytotoxic 50% aqueous MeOH fraction obtained during liquid−liquid fractionation yielded a dark brown solid (176.2 mg). A portion (100.0 mg) of this was subjected to gel permeation chromatography on a column of Sephadex LH-20 (4.0 g) in hexane/CH_2Cl_2 (1:4) and eluted with hexane/CH_2Cl_2 (1:4) (40 mL), CH_2Cl_2/acetone (3:2) (80 mL), and CH_2Cl_2/acetone (1:4) (40 mL). Twenty fractions (ca. 10 mL each) were collected and combined on the basis of their TLC patterns to yield two cytotoxic fractions, C (37.1 mg) and D (39.3 mg). Chromatography of fraction C (27.0 mg) on reversed-phase silica gel (0.5 g) and elution with increasing amounts of MeCN in H_2O afforded **6** (6.9 mg) and a further quantity of **5** (8.7 mg). Separation of fraction D (39.0 mg) as for fraction C afforded **6** (21.3 mg).

Dehydrocurvularin (4): pale yellow amorphous solid; comparison (TLC, ^{1}H NMR, ^{13}C NMR, and MS) with an authentic sample[8] confirmed its identity.

11-Methoxycurvularin (5): yellow oil, the identity of which was confirmed by comparison (TLC, ^{1}H NMR, ^{13}C NMR, and MS) with an authentic sample.[8]

11-Hydroxycurvularin (6): yellow oil; comparison (TLC, ^{1}H NMR, ^{13}C NMR, and MS) with an authentic sample[8] confirmed its identity.

Cultivation and Isolation of Metabolites of *As. cervinus* from the Rhizosphere of *A. thurberi*. For isolation of secondary metabolites, the fungus was cultured on PDA in 60 T-flasks for 28 days at 27 °C and processed as for *As. terreus* (see above) to yield the cytotoxic EtOAc extract (1.06 g). A portion (1.05 g) of this extract was suspended in 80% aqueous MeOH (100 mL) and extracted with hexane (3 × 200 mL). The aqueous MeOH layer was concentrated, and the cytotoxic residue (696 mg) was subjected to gel filtration over Sephadex LH-20 (5.0 g) and eluted with CH_2Cl_2/acetone (3:2) (200 mL) followed by MeOH (300 mL). Fractions were combined on the basis of their TLC patterns to furnish five combined fractions, A−E, of which fraction C was found to be the most cytotoxic. Chromatography of fraction A (92.0 mg) on silica gel (2.5 g) by elution with CH_2Cl_2/MeOH (100:1) afforded **8** (54.0 mg). Fraction C (25.0 mg) was further purified on silica gel (1.2 g) chromatography by elution with CH_2Cl_2/MeOH (20:1) to yield **9** (18.0 mg). Chromatography of fraction D (15.0 mg) on silica gel (0.3 g) by elution with hexane/acetone (1:1) afforded **7** (4.5 mg).

4*R,5*S**-Dihydroxy-3-methoxy-5-methylcyclohex-2-enone (7):** colorless semisolid; $[\alpha]^{20}_D$ +160.3° (*c* 0.01, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 250.5 (4.24) nm; IR (KBr) ν_{max} 3402, 2924, 2855, 1620, 1458, 1373, 1227, 1057 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.35 (1H, s, H-2), 4.32 (1H, s, H-4), 3.76 (3H, s, OCH_3-3), 2.68 (1H, d, *J* = 16.5 Hz, H-6a), 2.50 (1H, d, *J* = 16.5 Hz, H-6b), 1.34 (3H, s, CH_3-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 196.1 (C, C-1), 174.3 (C, C-3), 101.5 (CH, C-2), 74.5 (CH, C-4), 73.7 (C, C-5), 56.6 (CH_3, OCH_3-3), 48.6 (CH_2, C-6), 22.5 (CH_3, CH_3-5); APCIMS (+)-ve mode *m/z* 173 $[M + 1]^+$; APCIMS (−)-ve mode *m/z* 171 $[M - 1]^+$; HRFABMS: *m/z* 173.0811 $[M + 1]^+$ (calcd for $C_8H_{13}O_4$, 173.0814).

6-Methoxy-5(6)-dihydropenicillic acid (8): colorless oil; $[\alpha]^{20}_D$ +19.8° (*c* 0.19 $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 249.5 (4.46) nm; IR (KBr) ν_{max} 3356, 3132, 2932, 1751, 1643, 1342, 1219, 1034, 926 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.70 (1H, s, OH-4), 5.02 (1H, s, H-2), 3.87 (3H, s, OCH_3-3), 3.75 (1H, t, *J* = 10.0 Hz, H-6a), 3.49 (1H, d, *J* = 10.0 Hz, H-6b), 3.38 (3H, s, OCH_3-6), 2.46 (1H, m, H-5), 0.68 (3H, d, *J* = 7.0 Hz, CH_3-7); ^{13}C NMR (125 MHz, $CDCl_3$) 177.8 (C, C-3), 170.3 (C, C-1), 105.6 (C, C-4), 89.6 (CH, C-2), 75.0 (CH_2, C-6), 59.5 (CH_3, OCH_3-3), 59.2 (CH_3, OCH_3-6), 36.5 (CH, C-5), 11.1 (CH_3, C-7); APCIMS (+)-ve mode *m/z* 203 $[M + 1]^+$; HRFABMS *m/z* 203.0919 $[M + 1]^+$ (calcd for $C_9H_{15}O_5$, 203.0919).

Penicillic Acid (9): colorless crystalline solid; mp 79−80 °C (lit.[10c] 82−85 °C); IR, ^{1}H and ^{13}C NMR, and FABMS data consistent with literature values.[10]

Cultivation and Isolation of Metabolites of *As. wentii* from the Rhizosphere of *L. tridentata*. For isolation of bioactive compounds, the fungus was cultured in 20 T-flasks (800 mL) each containing 135 mL of PDA, for 28 days at 27 °C. Extraction of the culture medium with EtOAc and liquid−liquid fractionation of a portion (1.0 g) of the resulting extract (1.122 g) as for *As. terreus* (see above) afforded the cytotoxic $CHCl_3$ fraction (673 mg). A portion (630 mg) of this was subjected to gel permeation chromatography on a column of Sephadex LH-20 (15.0 g) in hexane/CH_2Cl_2 (1:4) and eluted with hexane/CH_2Cl_2 (1:4) (350 mL), CH_2Cl_2/acetone (3:2) (200 mL), CH_2Cl_2/acetone (1:4) (100 mL), CH_2Cl_2/MeOH (1:1) (100 mL), and MeOH (100 mL). Seventeen fractions (20 mL each) were collected while the column was eluted with hexane/CH_2Cl_2 (1:4). The last two fractions (fractions 16 and 17) eluted with hexane/CH_2Cl_2 (1:4) (182.0 mg) and the fractions eluted with CH_2Cl_2/acetone (3:2) (204.1 mg) were found to be cytotoxic. These fractions were combined, and a portion (30.0 mg) of it was purified by preparative TLC on silica gel (diethyl ether, double elution) to obtain **9** (22.0 mg), identical with the above obtained sample of penicillic acid.

Catalytic Hydrogenation of 9. A solution of **9** (6.0 mg) in EtOH (0.5 mL) containing Pd on carbon (10%, 1.5 mg) was stirred in an atmosphere of H_2 for 5 min (TLC control). The solution was filtered through a plug of cotton, and the solvent was evaporated under reduced pressure to afford 5(6)-dihydropenicillic acid (**16**) as a white solid (6.0 mg). Its ^{1}H NMR, ^{13}C NMR, and MS data were consistent with those reported in the literature.[13]

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References and Notes

(1) Studies on Arid Land Plants and Microorganisms, Part 5. For Part 4, see: Wijeratne, E. M. K.; Carbonezi, C.; Takahashi, J. A.; Seliga, C. J.; Turbyville, T. J.; Pierson, E. E.; Pierson, L. S., III; VanEtten, H. D.; Whitesell, L.; Bolzani, V. da S.; Gunatilaka, A. A. L. *J. Antibiot.* **2004**, *57*, 541−546.

(2) Gugnani, H. C. *Frontiers Biosci*. **2003**, *8*, 346−357.

(3) (a) Asai, A.; Yamashita, Y.; Ando, K.; Kakita, S.; Kita, K.; Suzuki, Y.; Mihara, A.; Ashizawa, T.; Mizukami, T.; Nakano, H. *J. Antibiot*. **1999**, *52*, 1046−1049. (b) Kaji, A.; Saito, R.; Nomura, M.; Miyamoto, K.-I.; Kiriyama, N. *Anticancer Res.* **1997**, *17*, 3675−3680. (c) Fang, F.; Vi, H.; Shiomi, K.; Masuma, R.; Yamaguchi, Y.; Zhang, C. G.; Zhang, X. W.; Tanak, Y.; Omura, S. *J. Antibiot*. **1997**, *50*, 919−925. (d) Bradner, W. T.; Bush, J. A.; Myllymaki, R. W.; Nettleton, D. E.; O'Herron, F. A. *Antimicrob. Agents Chemother*. **1975**, *8*, 159−163. (e) Schroeder, H. W.; Verrett, M. J. *Can. J. Microbiol*. **1969**, *15*, 895−898. (f) Kohno, J.; Hiramatsu, H.; Nihio, M.; Sakurai, M.; Okuda, T.; Komatsubara, S. *Tetrahedron* **1999**, *55*, 11247−11252. (g) Takahashi, C.; Yoshihira, K.; Natori, S.; Umeda, M. *Chem. Pharm. Bull*. **1976**, *24*, 613−620. (h) Hamasaki, T.; Kimura, Y. *Agric. Biol. Chem*. **1983**, *47*, 163−165. (i) Assante, G.; Camarda, L.; Nasini, G. *Gazz. Chim. Ital*. **1980**, *110*, 629−631. (j) Kondoh, M.; Usui, T.; Mayumi, T.; Osada, H. *J. Antibiot*. **1998**, *51*, 801−804. (k) Calton, G. J.; Ranieri, R. L.; Espenshade, M. A. *J. Antibiot*. **1978**, *31*, 38−42. (l) Ingber, D.; Fujita, T.; Kishimoto, S.; Sudo, K.; Kanamaru, T.; Brem, H.; Folkman, J. *Nature* **1990**, *348*, 555−557. (m) Ono, K.; Nakane, H.; Shimizu, S.; Koshimura, S. *Biochem. Biophys. Res. Commun*. **1991**, *174*, 56−62.

(4) Wijeratne, E. M. K.; Turbyville, T. J.; Zhang, Z.; Bigelow, D.; Pierson, L. S., III.; VanEtten, H. D.; Whitesell, L.; Canfield, L. M.; Gunatilaka, A. A. L. *J. Nat. Prod*. **2003**, *66*, 1567−1573.

(5) Zhou, G.-X.; Wijeratne, E. M. K.; Bigelow, D.; Zhang, Z.; Pierson, L. S., III; VanEtten, H. D.; Gunatilaka, A. A. L. *J. Nat. Prod*. **2004**, *67*, 328−332.

(6) (a) Kimura, Y.; Hamasaki, T.; Nakajima, H. *Tetrahedron Lett.* **1982**, *23*, 225−228. (b) Ellestad, G. A.; Mirando, P.; Kunstmann, M. P. *J. Org. Chem*. **1973**, *24*, 4204−4205.

(7) Lai, S.; Shizuri, Y.; Yamamura, S.; Kawai, K.; Yokohama, H. *Tetrahedron Lett*. **1989**, *30*, 2241−2244.

(8) Zhan, J.; Wijeratne, E. M. K.; Seliga, C. J.; Zhang, J.; Pierson, E. E.; Pierson, L. S., III; VanEtten, H. D.; Gunatilaka, A. A. L. *J. Antibiot.* **2004**, *57*, 341−344.

(9) Lai, S.; Shizuri, Y.; Yamaura, S.; Kawai, K.; Furukawa, H. *Bull. Chem. Soc. Jpn*. **1991**, *64*, 1048−1050.

(10) (a) Kakemi, K.; Sezaki, H.; Iwamoto, K.; Kobayashi, H.; Inui, K. *Chem. Pharm. Bull*. **1971**, *19*, 730−736. (b) Kimura, Y.; Nakahara, S.; Fujioka, S. *Biosci. Biotech. Biochem*. **1996**, *60*, 1375−1376. (c) Nimikoshi, M.; Neqishi, R.; Nagai, H.; Dmitrenok, A.; Kobayashi, H. *J. Antibiot*. **2003**, *56*, 755−761.

(11) Sassa, T.; Hayakaru, S.; Ikeda, M.; Miura, Y. *Agric. Biol. Chem*. **1971**, *35*, 2130−2131.

(12) Elsohly, H. N.; Slatkin, D. J.; Schiff, P. L. Jr.; Knapp, J. E. *J. Pharm. Sci.* **1974**, *63*, 1632−1633.

(13) (a) Varga, J.; Kevei, E.; Rinyu, E.; Teren, J.; Kozakiewicz, Z. *Appl. Environ. Microbiol*. **1996**, *G2*, 4461−4464. (b) Hamasaki, T.; Kimura, Y. *Agri. Biol. Chem*. **1983**, *47*, 163−165. (c) Assante, G.; Camarda, L.; Nasini, G. *Gazz. Chim. Ital*. **1980**, *110*, 629−631. (d) Assante, G.; Camarda, L.; Merlini, L.; Nasini, G. Abstracts of Papers, *11th IUPAC Int. Symp. Chem. Nat. Prod.*, Sofia, Bulgaria, 1978; Vol. 2, pp 171−172. (e) Assante, G.; Camarda, L.; Merlini, L.; Nasini, G. *Gazz. Chim. Ital*. **1979**, *109*, 151−153. (f) Wells, J. M.; Cole, R. J.; Kirksey, J. W. *Appl. Microbiol*. **1975**, *30*, 26−28. (g) Schroeder, H. W.; Varrett, M. J. *Can. J. Microbiol*. **1969**, *15*, 895−898.

(14) Alvi, K. A.; Pu, H.; Luche, M.; Rice, A.; App, H.; McMahon, G.; Dare, D.; Margolis, B. *J. Antibiot.* **1999**, *52*, 215−223.

(15) Hirada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy: Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983; Chapter 1, pp 1−31.

(16) Fujiwara, T.; Sato, A.; Kawamura, Y.; Matsumoto, K.; Itazaki, H. Japanese Patent No. JP6239852, 1994.

(17) Sekiguchi, J.; Katayama, S.; Yamada, Y. *App. Environ. Microbiol*. **1987**, *53*, 1531−1535.

(18) Rubinstein, L. V.; Shoemaker, R. H.; Paul, K. D.; Simon, R. M.; Tosini, S.; Skehan, P.; Scudiero, D. A.; Monks, A.; Boyd, M. R. *J. Nat. Cancer Inst*. **1990**, *82*, 1113−1118.

(19) Kobayashi, A.; Hino, T.; Yata, S.; Itoh, T. J.; Sato, H.; Kawazu, K. *Agric. Biol. Chem*. **1988**, *52*, 3119−3123.

(20) Matsushita, N.; Akinaga, S.; Agatsuma, T. International Patent No. WO 2004/024141, 2004 (pending).

(21) Whitesell, L.; Bagatell, R.; Falsey, R. *Curr. Cancer Drug Targets* **2003**, *3*, 349−358.

(22) (a) Stetina, R. *Folia Biol.* **1986**, *32*, 406−413. (b) Kawasaki, I.; Oki, T.; Umeda, M.; Saito, M. *Jpn. J. Exp. Med*. **1972**, *42*, 327−340. (c) Dickens, F.; Jones, H. E. H. *Brit. J. Cancer* **1963**, *17*, 100−108. (d) Larsen, J.; Olson, L. W. *J. Phytopath*. **1992**, *135*, 1−5. (e) Umeda, M.; Yamamoto, T.; Saito, M. *Jpn. J. Exp. Med*. **1972**, *42*, 527−535. (f) Bando, M.; Hasegawa, M.; Tsuboi, Y.; Miyake, Y.; Shiina, M.; Ito, M.; Handa, H.; Nagai, K.; Kataoka, T. *J. Biol. Chem*. **2003**, *278*, 5786−5793.

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