JOURNAL OF NATURAL PRODUCTS

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Nigerapyrones A−H, α-Pyrone Derivatives from the Marine Mangrove-Derived Endophytic Fungus *Aspergillus niger* MA-132

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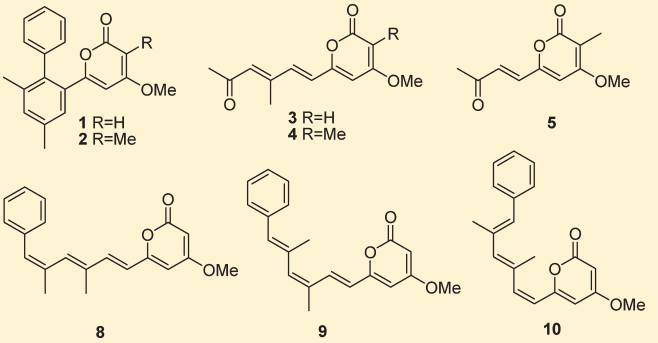
Supporting Information

ABSTRACT: Eight new α-pyrone derivatives, namely, nigerapyrones A−E (**1**−**5**) and nigerapyrones F−H (**8**−**10**), along with two known congeners, asnipyrones B (**6**) and A (**7**), were isolated from *Aspergillus niger* MA-132, an endophytic fungus obtained from the fresh tissue of the marine mangrove plant *Avicennia marina*. The structures of these compounds were elucidated on the basis of spectroscopic analysis. The undescribed geometries of the trisubstituted double bonds (C-9 and C-11) for asnipyrone B (**6**) have now been explicitly determined, while the incorrect placement of the methyl group at C-5 of asnipyrone A (**7**) has now been revised at C-3. The cytotoxic activities of the isolated α-pyrone derivatives against eight tumor cell lines as well as antimicrobial activities against two bacteria and four plant-pathogenic fungi of these compounds were evaluated. Compounds **2**, **4**, **5**, and **7** showed weak cytotoxicity against some of the tested tumor cell lines.



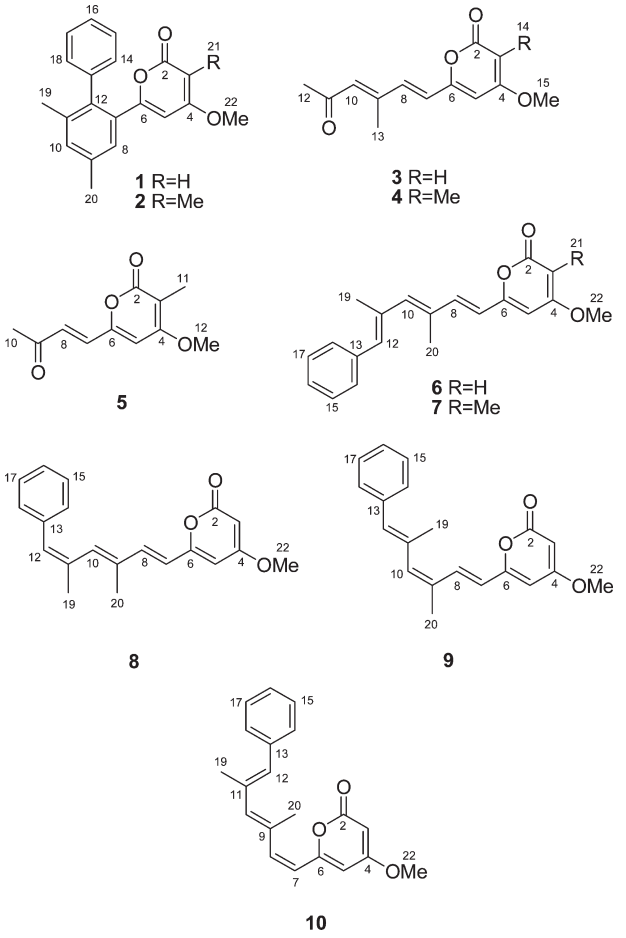
There are wide varieties of ecosystems in the vast marine environment including hitherto unexploited and taxonomically and geographically undescribed organisms. Marine microorganisms, especially marine fungi, are known to be rich sources of structurally interesting and biologically active compounds.[1−3] Chemical investigations of mangrove-derived endophytic fungi, especially those from the subtropical island of Hainan, China, have shown a sharp increase in recent years.[4−6] During our ongoing search for bioactive metabolites from marine-derived fungi,[7−13] a strain of *Aspergillus niger* MA-132, isolated from the inner tissue of the marine mangrove plant *Avicennia marina* collected at Dongzhai Harbor in Hainan, P. R. China, was cultured by shake-flask fermentation in Wicherhame medium. An organic extract of the culture medium led to the isolation of eight new α-pyrone derivatives, nigerapyrones A−E (**1**−**5**) and nigerapyrones F−H (**8**−**10**), as well as two known congeners, asnipyrones B (**6**) and A (**7**). The cytotoxicities against eight tumor cell lines and antimicrobial activities against two bacteria and four plant-pathogenic fungi of these compounds were evaluated. This paper describes the isolation, structure elucidation, and cytotoxic activities of the isolated compounds.

RESULTS AND DISCUSSION

The mycelia and culture broth of *A. niger* MA-132 were separated and were both extracted with EtOAc. The combined extracts were further purified by a combination of column chromatography including silica gel, Sephadex LH-20, Lobar LiChroprep RP-18, and semipreparative HPLC, to yield 10 α-pyrone derivatives (**1**−**10**).

Compound **1** was obtained as yellowish, amorphous powder. The molecular formula was determined from HREIMS data as $C_{20}H_{18}O_3$, implying 12 degrees of unsaturation. In examining the ^{1}H NMR data (Table 1 and Supporting Information), signals for one methoxy (H_3-22) and two tertiary (H_3-19 and H_3-20) methyls were observed in the higher field portion of the spectrum, two *meta*-coupled aromatic protons (H-3 and H-5) attributable to a pyrone unit were detected near δ_H 5.5 ppm, and seven aromatic protons, with two derived from a *meta*-coupled phenyl unit (H-8 and H-10) and five attributable from a monosubstituted phenyl moiety (H-14 through H-18), were

Received: May 3, 2011
Published: July 20, 2011



observed from δ_H 7 to 7.5 ppm. The ^{13}C NMR and DEPT spectra (Table 2 and Supporting Information) exhibited the presence of 18 carbon signals containing two overlapped signals, including three methyls (with one methoxy), nine aromatic methines, and eight quaternary (with seven aromatic and one carbonyl) carbon atoms. Exhaustive analyses of the NMR data of **1** showed some similarity to asnipyrone B, a phenyl-trienyl-α-pyrone derivative isolated from the fungal strain *A. niger* DSM 2182.[14] However, the two methines (CH-7 and CH-12) in asnipyrone B were replaced by two quaternary carbons in **1**. This was indicated by the fact that the two olefinic proton signals (H-7 and H-12) in asnipyrone B disappeared in the ^1H NMR spectrum of **1**. Accordingly, two olefinic methines (C-7 and C-12) of asnipyrone B were replaced by two aromatic quaternary carbon atoms in the ^{13}C NMR spectrum of **1**. The above evidence indicated that C-7 and C-12 were connected to each other and were part of a benzene ring in **1**. The HMBC correlations (Figure 1) from H-8 to C-6 and C-12, from H-14/H-18 to C-12, from H_3-19 to C-10 and C-12, and from H_3-20 to C-8 and C-10 confirmed the above deduction (Figure 1). The structure of compound **1** was therefore determined as 6-(4,6-dimethylbiphenyl-2-yl)-4-methoxy-2*H*-pyran-2-one, and the trivial name nigerapyrone A was assigned to this compound.

Compound **2**, a yellow, amorphous powder, was assigned the molecular formula $C_{21}H_{20}O_3$, having one CH_2 unit more than **1**, on the basis of HREIMS data. Detailed comparison of the NMR data of **2** with those of **1** suggested that compound **2** had the same basic structure as **1**. The methine signal resonating at δ_H 5.32 (H-3) and δ_C 88.4 (C-3) in the NMR spectra of **1** was absent in those of **2**. Instead, a quaternary carbon signal at δ_C 101.2 (C-3) and a methyl signal at δ_H 1.73 (H_3-21) and δ_C 8.7 (C-21) were observed. These observations suggested the assignment of a methyl group at C-3, which was verified by the HMBC correlations from H_3-21 to C-2 (δ_C 164.8), C-3, and C-4 (δ_C 165.9) (Scheme S1 in the Supporting Information). Thus, the structure of **2** was determined, and it was named nigerapyrone B.

Nigerapyrone C (**3**) has the molecular formula $C_{13}H_{14}O_4$ as determined by HREIMS data, with seven degrees of unsaturation. The ^1H NMR spectrum (Table 1) indicated the presence of two *meta*-coupled aromatic proton signals at δ_H 5.57 (d, J = 2.0 Hz, H-3) and 6.26 (d, J = 2.0 Hz, H-5), three olefinic protons at δ_H 6.57 (s, H-10), 6.75 (d, J = 15.8 Hz, H-7), and 7.04 (d, J = 15.8 Hz, H-8), and one methoxy group at δ_H 3.89 (15-OCH_3) and two methyl groups at δ_H 2.23 (s, H_3-12) and 2.25 (d, J = 1.0 Hz, H_3-13). The ^{13}C NMR and DEPT data (Table 2) exhibited the presence of 13 carbon signals. The structure of **3** was deduced from exhaustive analyses of the $^1H-^1H$ COSY and HMBC spectra. A ^3J correlation from H-7 to H-8 and a ^4J correlation from H-3 to H-5 were observed in the $^1H-^1H$ COSY experiment, while in the HMBC spectrum (Scheme S1 in the Supporting Information), a full set of all possible ^2J and ^3J correlations for H-3, H-5, H-7, and H-8 to the related carbons allowed for construction of the α-pyrone core structure as well as placing the side chain at C-6. Furthermore, HMBC correlations from H-10 to a sp^2 carbon C-9 and a carbonyl carbon C-11 suggested the presence of an α,β-unsaturated ketone in the side chain. Additionally, the correlation from H_3-12 to C-11 and from H_3-13 to C-8, C-9, and C-10 completed the assignment of the side chain. The large coupling constant (J = 15.8 Hz) for the olefinic protons H-7 and H-8 indicated the *E*-geometry for the double bond at C-7. The observed NOE correlations from H-5 to H-7, from H-7 to H_3-13, and from H-8 to H-10 established the *E*-geometry for the double bond at C-9.

The molecular formula of nigerapyrone D (**4**) was determined to be $C_{14}H_{16}O_4$ by HREIMS, having one more CH_2 unit than that of **3**. The ^1H and ^{13}C NMR spectroscopic data of **4** were similar to those of **3** with only some chemical shift differences at C-3. Compared with **3**, the NMR spectroscopic data (Tables 1 and 2) suggested the presence of an additional methyl group at C-3 in **4**, which was supported by the HMBC correlations from H_3-14 to C-2, C-3, and C-4 (Scheme S1 in the Supporting Information). The double-bond geometries of **4** were deduced to be the same as that of **3**, according to the NOESY experiment and coupling constants.

Nigerapyrone E (**5**) showed the molecular formula $C_{11}H_{12}O_4$ as determined by HREIMS with six degrees of unsaturation. The 1D NMR spectroscopic data also indicated the presence of the α-pyrone moiety with the side chain at C-6 and an α,β-unsaturated ketone in the side chain. In the HMBC spectrum, the observed cross-peaks from H_3-11 to C-2, C-3, and C-4 indicated the methyl group at C-3 (Scheme S1 in the Supporting Information). Moreover, HMBC correlations from H-5 to C-6 and C-7 and from H-7 and H-8 to C-6 confirmed the assignment of the side chain. The *E*-geometry for the double bond at C-7 was determined on the basis of the coupling constants (J = 15.9 Hz) of H-7 and H-8.

The HREIMS data of **6** gave a molecular formula of $C_{20}H_{20}O_3$, with 11 degrees of unsaturation, identical to that of asnipyrone B.[14] Although several NMR data for asnipyrone B were incorrectly assigned in the literature report (Table S1 in the Supporting Information), detailed analyses of the NMR data suggested that **6** was asnipyrone B. It is noteworthy to mention

Table 1. ^{1}H NMR Data for Compounds 1−5 and 8−10 (500 MHz, *J* in Hz)

position	1[a]	2[a]	3[a]	4[b]	5[a]	8[b]	9[b]	10[a]
3	5.32, d (2.2)		5.57, d (2.0)			5.45, d (2.5)	5.44, d (2.0)	5.49, d (2.0)
5	5.61, d (2.2)	5.87, s	6.26, d (2.0)	6.20, s	6.97, s	5.84, d (2.5)	5.88, d (2.0)	6.12, d (2.0)
7			6.75, d (15.8)	6.46, d (15.6)	7.17, d (15.9)	5.98, d (15.5)	6.10, d (15.5)	6.00, d (12.5)
8	7.32, br s	7.41, br s	7.04, d (15.8)	7.15, d (15.6)	6.80, d (15.9)	7.22, m	7.75, d (15.5)	6.52, d (12.5)
10	7.26, br s	7.28, br s	6.57, br s	6.35, br s	2.34, s	6.50, s	6.27, s	6.28, br s
11					1.88, s			
12			2.23, s	2.26, s	4.01, s	6.46, s	6.46, s	6.60, br s
13			2.25, d (1.0)	2.27, d (1.0)				
14	7.17, d (6.9)	7.22, d (7.4)		1.96, s		7.26, m	7.34, m	7.37, m
15	7.39, t (7.2)	7.44, t (7.4)	3.89, s	3.90, s		7.34, m	7.34, m	7.37, m
16	7.32, t (7.4)	7.36, t (7.4)				7.18, m	7.25, m	7.24, m
17	7.39, t (7.2)	7.44, t (7.4)				7.34, m	7.34, m	7.37, m
18	7.17, d (6.9)	7.22, t (7.4)				7.26, m	7.34, m	7.37, m
19	2.06, s	2.09, s				2.08, s	2.09, s	2.08, d (1.2)
20	2.39, s	2.41, s				1.74, s	2.00, s	2.09, d (1.1)
21		1.73, s						
22	3.74, s	3.51, s				3.81, s	3.79, s	3.88, s

[a] Measured in acetone-d_6. [b] Measured in $CDCl_3$.

Table 2. ^{13}C NMR Data for Compounds 1−5 and 8−10 (125 MHz)

position	1[a]	2[a]	3[a]	4[b]	5[a]	8[b]	9[b]	10[a]
2	163.6, C	164.8, C	163.1, C	164.3, C	163.7, C	164.1, C	163.9, C	163.5, C
3	88.4, CH	101.2, C	90.3, CH	104.5, C	106.2, C	88.4, CH	88.6, CH	88.9, CH
4	171.3, C	165.9, C	171.6, C	165.1, C	165.7, C	171.2, C	171.0, C	171.7, C
5	103.4, CH	99.2, CH	103.8, CH	97.8, CH	102.8, CH	100.6, CH	100.4, CH	102.9, CH
6	162.4, C	160.1, C	158.8, C	156.3, C	155.5, C	159.3, C	159.5, C	159.9, C
7	133.2, C	133.2, C	126.2, CH	124.9, CH	133.2, CH	117.6, CH	119.6, CH	119.7, CH
8	127.8, CH	127.7, CH	138.7, CH	138.1, CH	130.8, CH	141.0, CH	134.5, CH	142.8, CH
9	137.8, C	137.8, C	147.8, C	147.6, C	197.2, C	134.3, C	131.8, C	134.4, C
10	133.6, CH	133.5, CH	131.4, CH	130.4, CH	28.4, CH_3	137.8, CH	140.3, CH	139.4, CH
11	138.0, C	138.1, C	199.0, C	199.0, C	9.3, CH_3	134.3, C	134.8, C	135.8, C
12	138.8, C	138.7, C	32.1, CH_3	32.1, CH_3	57.3, CH_3	130.9, CH	133.2, CH	132.8, CH
13	140.4, C	140.9, C	13.7, CH_3	13.6, CH_3		137.8, C	137.5, C	138.6, C
14	130.4, CH	130.6, CH		8.9, CH_3		128.7, CH	129.3, CH	130.0, CH
15	129.2, CH	129.5, CH	56.8, CH_3	56.3, CH_3		128.2, CH	128.2, CH	129.0, CH
16	128.0, CH	128.1, CH				126.7, CH	126.8, CH	127.6, CH
17	129.2, CH	129.5, CH				128.2, CH	128.2, CH	129.0, CH
18	130.4, CH	130.6, CH				128.7, CH	129.3, CH	130.0, CH
19	20.8, CH_3	20.9, CH_3				24.8, CH_3	19.0, CH_3	18.8, CH_3
20	21.0, CH_3	21.0, CH_3				13.9, CH_3	20.9, CH_3	18.5, CH_3
21		8.7, CH_3						
22	56.5, CH_3	56.7, CH_3				55.6, CH_3	55.8, CH_3	56.6, CH_3

[a] Measured in acetone-d_6. [b] Measured in $CDCl_3$.

that the geometries for the trisubstituted double bonds (C-9 and C-11) for asnipyrone B were not discussed in the reference.[14] For **6**, both of the *E*-geometries for these two double bonds were unambiguously determined by the observed NOE correlations from H-10 to H-8 and H_3-19 and from H_3-20 to H-7 and H-12 (Figure 2).

Compound **7** was assigned the molecular formula $C_{21}H_{22}O_3$, identical to that of asnipyrone A,[14] on the basis of HRESIMS data. Detailed comparison of the NMR data (Table S1 in the Supporting Information) suggested that compound **7** had the same structure as asnipyrone A. However, the position of the methyl group C-21 was incorrectly assigned at C-5 in the earlier report.[14] The HMBC correlations from H_3-21 to C-2, C-3, and C-4 indicated that this methyl group should be attached at C-3 (Figure 1). The results from NMR data prediction by ChemDraw (Ultra 8.0) also supported the placement of the methyl group at C-3, rather than at C-5. The large coupling constant (15.5 Hz) indicated the *E*-geometry for the double bond at C-7. NOE correlations from H-10 to H-8 and H_3-19 and from H_3-20 to H-7 and H-12 established the *E*-geometry for the double bonds at C-9 and C-11 (Figure 2).

dx.doi.org/10.1021/np200381u |*J. Nat. Prod.* 2011, 74, 1787–1791

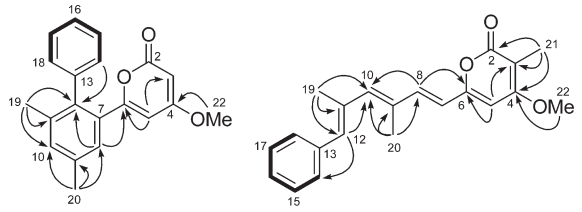


Figure 1. Key HMBC (arrows) and ^{1}H−^{1}H COSY (bold lines) correlations of compounds **1** and **7**.

Exhaustive analyses of HREIMS and NMR data as well as comparison with those of **6** revealed that compounds **8**−**10** had the same molecular formula $C_{20}H_{20}O_3$ and possess the same planar structure as **6**. However, the double-bond geometries were different. For compounds **8** and **9**, the *E*-geometry for the double bond at C-7 was evident from the large coupling constant (15.5 Hz) for $J_{7,8}$. In the NOESY spectrum of **8**, the correlations from H-8 to H-10, from H_3-19 to H-12, and from H_3-20 to H-7 and H_3-19 obviously established the *E*- and *Z*-geometries for the double bonds at C-9 and C-11, respectively. In the NMR spectra of **9**, however, the NOE correlations from H-10 to H-12 and H_3-20, from H_3-19 to H-8, and from H_3-20 to H-7 and H-10 clearly indicated *Z*- and *E*-geometries for the double bonds at C-9 and C-11, respectively, which were opposite those of **8**. As for compound **10**, the smaller coupling constant (12.5 Hz) for $J_{7,8}$ as well as the NOE correlations from H-10 to H-8 and H_3-19 indicated the *Z*-geometry for the double bond at C-7. Additionally, the NOE correlations from H-10 to H-8 and H_3-19 and from H_3-20 to H-12 indicated the *E*-geometry for the double bonds at C-9 and at C-11. The structures of **8**−**10** were assigned and named nigerapyrones F−H, respectively.

The biological activities of compounds **1**−**10** were examined by cytotoxicity and antimicrobial bioassays. The cytotoxicities against DU145, HeLa, HepG2, MCF-7, NCI-H460, A549, MDA-MB-231, and SW1990 tumor cell lines were determined. Nigerapyrone E (**5**) showed cytotoxicities against SW1990, MDA-MB-231, and A549 cell lines with IC_{50} values of 38, 48, and 43 μM, respectively, which were stronger than that of the positive control, fluorouracil (see Experimental Section). This compound also showed weak or moderate activity against MCF-7, HepG2, Du145, NCI-H460, and MDA-MB-231 cell lines with IC_{50} values of 105, 86, 86, 43, and 48 μM, respectively. Nigerapyrone B (**2**) showed selective activity against the HepG2 cell line with an IC_{50} of 62 μM, while asnipyrone A (**7**) showed activity against the A549 cell line with an IC_{50} of 62 μM, and nigerapyrone D (**4**) showed moderate or weak activity against the MCF-7, HepG2, and A549 cell lines, with IC_{50} values of 121, 81, and 81 μM, respectively. In the antimicrobial assay, no obvious activity could be observed for these compounds against the six tested microbial strains.

EXPERIMENTAL SECTION

General Experimental Procedures. UV spectra were measured on a PuXi TU-1810 UV−visible spectrophotometer. 1D and 2D NMR spectra were recorded at 500 and 125 MHz for ^{1}H and ^{13}C, respectively, on a Bruker Avance 500 MHz spectrometer with TMS as internal standard. Mass spectra were determined on a VG Autospec 3000 or an API QSTAR Pulsar 1 mass spectrometer. Column chromatography (CC) was performed with silica gel (200−300 mesh, Qingdao Haiyang Chemical Co.), Lobar LiChroprep RP-18 (40−63 μm; Merck), and Sephadex LH-20 (18−110 μm, Merck). Semipreparative HPLC was performed using a Dionex HPLC system equipped with a P680 pump, an ASI-100 automated sample injector, and a UVD340U multiple wavelength detector controlled using Chromeleon software, version 6.80.

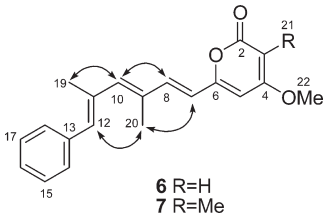


Figure 2. Key NOESY correlations of compounds **6** and **7**.

Fungal Material. The fungus *Aspergillus niger* MA-132 was isolated from a fresh, healthy sample of the mangrove plant *Avicennia marina* collected at Dongzhai Harbor in Hainan, P. R. China, in August 2004. The fungus was identified by analysis of the ITS region of the rDNA, as described in our previous report.[13] The sequence data derived from the fungal strain have been deposited at GenBank with the accession number HQ891666. The BLAST result showed that the sequence was the most similar (99%) to the sequence of *A. niger* (compared with GU338398.1, GI 284795036). The strain is preserved at the Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences.

Fermentation, Extraction, and Isolation. For the isolation and identification of new metabolites, mass growth of the fungus was accomplished by incubation on a rotary shaker (160 rpm) at 28 °C for 7 days in 120 × 500 mL conical flasks containing liquid medium (200 mL/flask) composed of dextrose (20 g/L), malt extract (5 g/L), peptone (10 g/L), and seawater, pH 7.2−7.4.

The whole fermented cultures (24 L) were filtered to separate the broth from the mycelia. The former was extracted three times with EtOAc, while the latter was extracted three times with a mixture of 80% acetone and 20% H_2O. The acetone solution was evaporated under reduced pressure to afford an aqueous solution, which was then extracted three times with EtOAc. Because the TLC and HPLC profiles of the two EtOAc solutions were almost identical, they were combined and concentrated under reduced pressure to give an extract (10.0 g) for further separation.

The extract was fractionated by silica gel vacuum liquid chromatography (VLC) using different solvents of increasing polarity from petroleum ether (PE) to MeOH to yield 14 fractions (Frs. 1−14) based on TLC analysis. Fr. 5 (390 mg) was further purified by CC over silica gel eluting with a $CHCl_3$/MeOH gradient (from 0:1 to 1:1) and by semipreparative HPLC (Elite ODS-BP column, 10 μm; 10.0 × 300 mm; 80% MeOH/H_2O, 3 mL/min) to afford **6** (3.0 mg, t_R 22.0 min), **7** (20.2 mg, t_R 25.9 min), **8** (3.0 mg, t_R 18.0 min), **9** (3.4 mg, t_R 20.3 min), and **10** (3.0 mg, t_R 17.0 min). Fr. 6 (328 mg) was purified by Sephadex LH-20 (MeOH) to afford two subfrations, Fr. 6-1 (80.0 mg) and Fr. 6-2 (34.0 mg). Fr. 6-1 was further purified by semipreparative HPLC (60% MeOH/H_2O, 3 mL/min) to yield **3** (3.0 mg, t_R 15.3 min), **4** (5.4 mg, t_R 18.0 min), and **5** (2.8 mg, t_R 11.4 min). Fr. 6-2 was also purified by semipreparative HPLC, eluting with 80% MeOH/H_2O, to provide compounds **1** (3.2 mg, t_R 11.4 min) and **2** (4.0 mg, t_R 13.0 min).

*Nigerapyrone A (**1**):* yellowish, amorphous powder; UV (MeOH) λ_{max} (log ε) 203 (4.66), 301 (4.10) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 306 $[M]^+$ (100), 289 $[M - H_2O + H]^+$ (26), 278 $[M - CO]^+$ (41), 246 $[M - CH_3COOH]^+$ (77), 231 $[M - CH_3COOH - CH_3]^+$ (61); HREIMS *m/z* 306.1264 $[M]^+$ (calcd for $C_{20}H_{18}O_3$, 306.1256).

*Nigerapyrone B (**2**):* yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 203 (4.48), 323 (3.97) nm; ^{1}H and ^{13}C NMR data, see Tables 1

and 2; EIMS m/z 320 $[M]^+$ (100), 303 $[M - H_2O + H]^+$ (13), 292 $[M - CO]^+$ (28), 275 $[M - COOH]^+$ (34), 260 $[M - CH_3COOH]^+$ (40), 245 $[M - CH_3COOH - CH_3]^+$ (71); HREIMS m/z 320.1417 $[M]^+$ (calcd for $C_{21}H_{20}O_3$, 320.1412).

Nigerapyrone C (**3**): yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 215 (3.85), 266 (3.87), 346 (3.89) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS m/z 234 $[M]^+$ (80), 219 $[M - CH_3]^+$ (16), 191 $[M - CH_3CO]^+$ (43), 125 $[M - C_7H_9O]^+$ (98), 109 $[M - C_6H_5O_3]^+$ (100); HREIMS m/z 234.0890 $[M]^+$ (calcd for $C_{13}H_{14}O_4$, 234.0892).

Nigerapyrone D (**4**): yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 217 (3.92), 272 (3.94), 371 (3.85) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS m/z 248 $[M]^+$ (100), 233 $[M - CH_3]^+$ (24), 205 $[M - CH_3CO]^+$ (50), 139 $[M - C_7H_9O]^+$ (67), 109 $[M - C_7H_7O_3]^+$ (90); HREIMS m/z 248.1052 $[M]^+$ (calcd for $C_{14}H_{16}O_4$, 248.1049).

Nigerapyrone E (**5**): yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 240 (4.12), 285 (3.55), 353 (3.74) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS m/z 208 $[M]^+$ (100), 193 $[M - CH_3]^+$ (29), 180 $[M - CO]^+$ (67), 165 $[M - CH_3CO]^+$ (68); HREIMS m/z 208.0739 $[M]^+$ (calcd for $C_{11}H_{12}O_4$, 208.0736).

Nigerapyrone F (**8**): yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 243 (4.59), 283 (4.29), 359 (4.48) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS m/z 308 $[M]^+$ (12), 293 $[M - CH_3]^+$ (3), 125 $[M - C_{14}H_{15}]^+$ (100); HREIMS m/z 308.1408 $[M]^+$ (calcd for $C_{20}H_{20}O_3$, 308.1412).

Nigerapyrone G (**9**): yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 241 (4.54), 283 (4.52), 369 (4.48) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS m/z 308 $[M]^+$ (42), 293 $[M - CH_3]^+$ (5), 183 $[M - C_6H_5O_3]^+$ (16), 125 $[M - C_{14}H_{15}]^+$ (100); HREIMS m/z 308.1414 $[M]^+$ (calcd for $C_{20}H_{20}O_3$, 308.1412).

Nigerapyrone H (**10**): yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 215 (4.41), 284 (4.36), 361 (4.42) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS m/z 308 $[M]^+$ (53), 293 $[M - CH_3]^+$ (22), 183 $[M - C_6H_5O_3]^+$ (53), 125 $[M - C_{14}H_{15}]^+$ (98), 69 $[M - C_{14}H_{15} - 2CO]^+$ (100); HREIMS m/z 308.1418 $[M]^+$ (calcd for $C_{20}H_{20}O_3$, 308.1412).

Cytotoxicity Assay. The cytotoxic activities against DU145 (human prostate carcinoma), HeLa (human epithelial carcinoma), HepG2 (human hepatocellular liver carcinoma), MCF-7 (human breast adenocarcinoma), NCI-H460 (human non-small-cell lung cancer), MDA-MB-231 (human breast cancer), SW1990 (human pancreatic cancer), and A549 (human lung carcinoma) cell lines were determined according to previously reported methods.[15] Fluorouracil was used as the positive control against tumor cell lines of A549, HepG2, DU145, MCF-7, SW1990, NCI-H460, and MDA-MB-231, with IC_{50} values of 52, 109, 3.3, 31, 121, 8.5, and 59 μM, respectively.

Antimicrobial Activity. Antimicrobial assays against two bacteria (*Staphylococcus aureus* and *Escherichia coli*) and four plant-pathogenic fungi (*Alternaria brassicae*, *Fusarium oxysporum*, *Coniella diplodiella*, and *Physalospora piricola*) were carried out using the disk diffusion method.[16] Chloramphenicol and amphotericin B were used as positive controls against bacteria and fungi, respectively.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. HMBC correlations for compounds **1**−**10** (Scheme S1), fully assigned NMR data of compounds **6** and **7** as well as those reported data for asnipyrones B and A in ref 14 (Table S1), and selected 1D and 2D NMR spectra of compounds **1**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

Financial support from the Natural Science Foundation of China (30910103914) and from the Ministry of Science and Technology of China (2010CB833800) is gratefully acknowledged.

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■ NOTE ADDED AFTER ASAP PUBLICATION

This paper was published on the Web on July 20, 2011, with missing footnotes in Tables 1 and 2. The corrected version was reposted on July 29, 2011.