Arenarins A−C: New Cytotoxic Fungal Metabolites from the Sclerotia of *Aspergillus arenarius*

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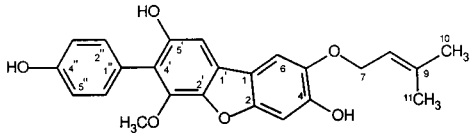
Received January 14, 1998

Three new terphenyl-type metabolites, arenarins A−C (**1**−**3**), have been isolated from the sclerotia of *Aspergillus arenarius* (NRRL 5012). The structures of these compounds were elucidated by analysis of 1-D and 2-D NMR data. Arenarins A−C exhibited mild activity in feeding assays against the dried-fruit beetle *Carpophilus hemipterus* and cytotoxicity against human tumor cell lines.

Certain fungi produce long-term survival structures called sclerotia. Our continuing investigations of *Aspergillus* spp. sclerotia as sources of new antiinsectan and other bioactive metabolites[1] have led to the isolation of three new prenylated terphenyl-type metabolites from the sclerotia of *Aspergillus arenarius* Raper et Fennell (Trichocomaceae) (NRRL 5012), which we have named arenarins A (**1**), B (**2**), and C (**3**). Details of this work are described here.



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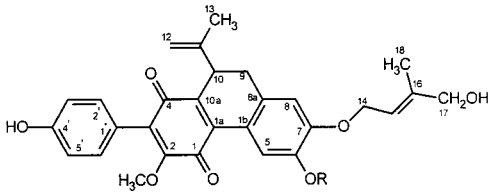
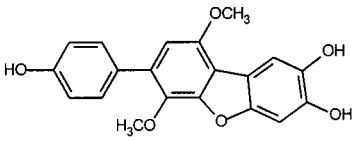


2 R = H
3 R = CH_3



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Sclerotia of *A. arenarius* were produced by solid substrate fermentation on corn kernels.[2] The CH_2Cl_2 extract of the sclerotia exhibited potent antiinsectan activity in feeding assays[2] against the agriculturally important corn pest *Helicoverpa zea* and the fungivorous dried fruit beetle *Carpophilus hemipterus* and was selected for further chemical investigation. The extract was fractionated using Sephadex LH-20 column chromatography, preparative TLC, and reversed-phase HPLC to afford arenarins A−C (**1**−**3**).

On the basis of HRFABMS and ^{13}C NMR data, the molecular formula of the major component (**1**) was assigned as $C_{24}H_{22}O_6$, requiring 14 unsaturation equivalents. The DEPT data revealed 19 protons bound to carbon, requiring three exchangeable protons in the molecule. The ^{13}C NMR spectrum contained 20 resonances in the olefinic/aromatic region. The ^1H and ^{13}C NMR data were consistent with the presence of a methoxy group, a 1-oxy-3-methyl-2-butenyl unit, an oxygenated 1,4-disubstituted benzene ring, a 1,2,4,5-tetrasubstituted benzene ring, and a pentasubstituted benzene ring. The ^{13}C NMR chemical shifts of the latter two units implied that each ring has three oxygen substituents in a 1,2,4-orientation. These structural units accounted for 13 unsaturation equivalents. The remaining unsaturation equivalent must be accounted for by a fourth ring.

^1H and ^{13}C NMR assignments and the terphenyl-type core structure of **1** were determined on the basis of HMQC and HMBC data (Table 1) and comparison to the known compound candidusin A (**4**), which possesses the same ring system as **1**.[3] An HMBC correlation of H-2″/H-6″ of the oxygenated 1,4-disubstituted benzene unit with C-4′ of the pentasubstituted ring required attachment of the 1,4-disubstituted ring to the pentasubstituted ring at C-4′. The pentasubstituted benzene ring was then connected to the 1,2,4,5-tetrasubstituted ring at the position shown in **1** on the basis of an HMBC correlation of H-6′ with C-1. The locations of the substituents in compound **1** were determined primarily on the basis of ^{13}C NMR, HMBC, and NOESY data. For example, the methoxy proton signal (H_3-3′, δ 3.98) correlated with the carbon signal at δ 142.3 (C-3′), and the chemical shift of C-3′ required its location ortho to another oxygenated substituent. The oxygenated methylene signal (H_2-7, δ 4.66) of the prenyl substituent correlated with the most upfield-shifted oxygenated carbon of the C-ring (C-5; δ 142.8). NOESY correlations of the signal for H-6 with the side-chain vinylic proton (H-8) and the isolated aromatic proton H-6′ located the oxygenated prenyl unit at C-5 and required the relative orientation of H-6 and H-6′ as shown in **1**. The proposed regiochemistry and the locations of the hydroxy groups

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S0163-3864(98)00001-9 CCC: $15.00
Published on Web 04/17/1998

Table 1. NMR Spectral Data for Arenarin A (**1**)

C/H	^{1}H[a] δ (mult; J_{H-H} in Hz)	^{13}C[b]	HMBC correlations[c]
1		115.6	
2		147.0	
3	7.11 (s)	98.2	1, 2, 4, 5
4		151.9	
5		142.8	
6	7.28 (s)	103.2	1′, 2, 4, 5
7	4.66 (br d, 6.9)	66.7	5, 8, 9
8	5.53 (m)	119.1	10, 11
9		139.3	
10	1.81 (s)	25.8	8, 9, 11
11	1.77 (s)	18.3	8, 9, 10
1′		126.4	
2′		142.1	
3′		142.3	
4′		117.9	
5′		149.4	
6′	7.06 (s)	98.9	1, 2′, 4′, 5′
1″		124.7	
2″/6″	7.29 (d, 8.4)	132.3	4′, 4″, 2″/6″
3″/5″	6.97 (d, 8.4)	116.3	1″, 4″, 3″/5″
4″		155.7	
4-OH[d]	9.40 (s)		3, 4, 5
3′-OCH$_3$	3.98 (s)	60.7	3′
5′-OH[d]	9.12 (s)		4′, 5′, 6′
4″-OH[d]	9.33 (s)		3″, 4″, 5″

[a] 300 MHz in $CDCl_3$. [b] 75 MHz in $CDCl_3$. [c] 600 MHz in $CDCl_3$ (^{1}H-dimension). [d] observed in DMSO-d_6.

were confirmed by observation of HMBC correlations of the three phenolic OH protons with nearby aromatic carbons when the data were recorded DMSO-d_6 (Table 1). For example, the phenolic OH proton at δ 9.40 correlated with C-3, C-4, and C-5, confirming the proposed locations of the oxygenated prenyl unit and the OH group at C-4 and C-5, respectively. The ^{1}H and ^{13}C NMR assignments for C-10 and C-11 were made on the basis of the upfield chemical shift of C-11 (δ 18.3) and NOESY correlations of H-7 with H-11 and of H-8 with H-10. Since one unsaturation equivalent remained to be accounted for, C-2 and C-2′ must be connected via an ether linkage through the remaining oxygen atom to complete the gross structure of **1**.

The molecular formula of arenarin B (**2**) was determined to be $C_{29}H_{28}O_7$ (16 unsaturations) by analysis of ^{13}C NMR and HRFABMS data, and DEPT results indicated that three exchangeable protons (hydroxy groups) were present. The NMR spectra (Table 2) contained signals corresponding to an oxygenated 1,4-disubstituted aromatic ring, a 1,4-dioxy-3-methyl-2-butenyl unit, a methoxy group, and two isolated aromatic protons, suggesting a structure related to that of **1**. In addition to these data, signals for an isoprene-derived 3,4-disubstituted 2-methyl-1-butenyl unit and two carbonyl groups characteristic of a quinone (^{13}C NMR signals at δ 186.1 and δ 183.5) were present.[4] These features accounted for all but one unsaturation equivalent, which must represent an additional ring. The terphenyl-type core structure of **2** was confirmed by analysis of HMBC data, and HMBC correlations for H_2-9 and H-10 provided all of the correlations necessary to establish attachment of the substituted butenyl unit to the terphenyl core structure. The C-10 methine proton signal (δ 3.78) showed HMBC correlations with C-4 (δ 186.1) and C-1a (δ 135.7) requiring attachment of C-10 to C-10a. HMBC correlations of the C-9 methylene protons (δ 2.86 and δ 3.03) to C-1b, C-8, C-8a, and C-10a required the attachment of C-9 to C-8a and supported the linkage of C-10 to C-1a. HMBC results also established the positions of the remaining substituents in compound **2**. HMBC correlation of H_2-14 to C-7 indicated that the 1,4-dioxygenated prenyl unit is connected to C-7 via an ether linkage. The upfield chemical shift of C-18 (δ 14.1) placed this group cis to the oxygenated methylene group (C-14) and established the *E* geometry for the C15−C16 double bond. The phenolic OH groups were located at C-6 and C-4′ by HMBC correlations (DMSO-d_6) from the OH proton signals to C-5, C-6, and C-7 and to C-3′/C-5′, and C-4′, respectively (Table 2). The methoxy group was positioned ortho to C-1 rather than ortho to C-4 on the basis of biogenetic similarity with **1** and the upfield chemical shift of C-1 relative to that of C-4. Thus, the structure of arenarin B was assigned as shown in **2**.

Table 2. NMR Spectral Data for Arenarin B (**2**) and Arenarin C (**3**)

	^{1}H[a] δ (mult; J_{H-H} = Hz)		^{13}C[b]		HMBC correlations[c]
C/H	**2**	**3**	**2**	**3**	**2**
1			183.5	184.0	
1a			135.7	135.3	
1b			121.7	121.2	
2			154.8	154.6	
3			127.9	127.5	
4			186.1	186.2	
5	7.72 (s)	7.77 (s)	115.7	113.0	1a, 6, 7, 8a
6			147.0	149.5	
7			144.2	147.7	
8	6.69 (s)	6.67 (s)	112.0	112.7	1b, 6, 7, 9
8a			129.6	130.3	
9	2.86 (d, 15.5)	2.8 (d, 15.6)	31.9	31.8	1b, 8, 8a, 10, 10a, 11
	3.03 (dd, 15.5, 7.2)	3.04 (dd, 15.6, 7.4)			
10	3.78 (d, 7.2)	3.78 (d, 7.4)	36.8	36.8	1a, 4, 9, 12, 13
10a			139.9	139.9	
11			142.5	142.6	
12	4.47 (s)	4.46 (s)	112.1	112.2	10, 13
	4.65 (s)	4.64 (s)			
13	1.72 (s)	1.69 (s)	21.8	21.8	10, 11, 12
14	4.69 (m)	4.68 (d, 7.2)	65.2	65.2	7, 15, 16
15	5.79 (m)	5.79 (m)	118.8	119.7	17, 18
16		141.0	140.2		
17	4.11 (s)	4.07 (d; 4.5)	67.5	67.8	15, 16
18	1.78 (s)	1.76 (s)	14.1	14.1	15, 16, 17
1′			122.7	122.6	
2′/6′	7.25 (d, 8.7)	7.24 (d, 8.7)	132.3	132.3	3, 4′, 2′/6′
3′/5′	6.86 (d, 8.7)	6.86 (d, 8.7)	114.8	114.9	1′, 4′, 3′/5′
4′			155.8	156.0	
2-OCH$_3$	3.85 (s)	3.90 (s)	65.1	61.2	2
6-OCH$_3$		3.76 (s)		56.1	
4′-OH[d]	9.62 (s)				3′, 4′, 5′
6-OH[d]	8.91 (s)				5, 6, 7

[a] 300 MHz in $CDCl_3$. [b] 75 MHz in $CDCl_3$. [c] 600 MHz in $CDCl_3$ (^{1}H-dimension). [d] Observed in DMSO-d_6.

The ^{1}H and ^{13}C NMR spectra (Table 2) for arenarin C (**3**) contained signals that matched very closely with those of compound **2**, differing only in the presence of an additional OMe group (δ 3.76 and δ 56.1; Table 2). These data suggested simple replacement of an OH group in **2** with an OMe group in **3**. This proposal was consistent with the HRFABMS data for **3**, which indicated a molecular formula of $C_{30}H_{30}O_7$. ^{13}C NMR chemical shift comparisons of the data for **2** and **3** in the olefinic/aromatic region suggested location of the new OMe group at C-6 rather than C-4′ (Table 2). This suggestion was confirmed by a strong NOESY correla-

tion of the new OMe signal with that of H-5. Complete ^{1}H and ^{13}C NMR assignments for compound **3** were established on the basis of spectral comparison with compound **2**.

Approximately 20 members of the terphenyl structural class have been previously reported as fungal metabolites.[5−7] In general, the reported metabolites contain terphenyl or terphenylquinone core structures with OH, MeO, or acetate substituents. To our knowledge, arenarins A−C are the first compounds of this structural class having prenyl group substituents, and arenarins B and C are the first examples possessing an additional carbocyclic ring attached to the terphenylquinone core structure.

Compounds **1**−**3** showed mild activity in feeding assays against the fungivorous beetle *Carpophilus hemipterus*. Arenarin A (**1**) caused a 13% reduction in feeding rate in assays against *C. hemipterus* adults and larvae relative to controls at a 100 ppm dietary level. Arenarins B and C (**2** and **3**) also induced some feeding reduction in assays against *C. hemipterus* adults (20% and 13%, respectively). Arenarins A and B (**1** and **2**) also showed cytotoxicity against human tumor cells in the NCI's 60-cell line panels, displaying average GI_{50} values of 4.8 and 3.8 μg/mL, respectively.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR data were obtained at 300 and 75.5 MHz, respectively. HMBC, HMQC, and NOESY experiments were recorded at 600 MHz (^{1}H-dimension), and HMBC and HMQC experiments were optimized for $^nJ_{CH} = 8$ Hz and $^1J_{CH} = 152$ Hz, respectively. Spectra were recorded in $CDCl_3$ or DMSO-d_6, and chemical shifts were referenced using the corresponding solvent signals (δ 7.24/δ 77.0 or δ 2.49/δ 39.5, respectively). Multiplicities of all carbon signals were verified through DEPT experiments. HPLC separations employed a Beckman Ultrasphere ODS column (5 μm particles, 250 × 10 mm; flow rate = 2.0 mL/min; UV monitoring at 215 nm). Low-resolution EI mass spectra were acquired at 70 eV using a VG Trio 1 quadrupole mass spectrometer equipped with a direct inlet probe. Low- or high-resolution FAB mass spectra were obtained using a VG ZAB-HF double-focusing mass spectrometer. Details of bioassays and other general experimental procedures have been described elsewhere.[2,8]

Isolation and Characterization of 1−3. The isolate of *A. arenarius* used in this study was originally obtained by E. Yuill from soil collected in Mysore, India. This culture was deposited in the USDA National Center for Agricultural Utilization Research and assigned the accession number NRRL 5012. Sclerotia of *A. arenarius* were produced by solid−substrate fermentation on corn kernels using general procedures described previously.[2] Ground sclerotia (67 g) were sequentially extracted with hexane, CH_2Cl_2, and CH_3OH. The CH_2Cl_2 extract (1.24 g) was triturated with a small amount of hexane (2 mL) and filtered to afford 320 mg of hexane-insoluble solid. This material was fractionated by Sephadex LH-20 column chromatography, eluting with CH_2Cl_2−hexane (4:1), followed by CH_2Cl_2−acetone (3:2) and CH_2Cl_2−acetone (1:4). Fractions were pooled on the basis of TLC behavior to afford 18 fractions. On the basis of their ^{1}H NMR spectra, fractions containing **1** and **2**, eluted with CH_2Cl_2−acetone (3:2), were separated further by preparative TLC (CH_2Cl_2−EtOAc 3:2) and reversed-phase HPLC (CH_3OH−H_2O 70:30 or 67:23) to yield arenarin A (5 mg) and arenarin B (3.8 mg). Fractions containing **3**, eluted with CH_2Cl_2−acetone 3:2, were separated further by reversed-phase HPLC (CH_3OH−H_2O 72:28) to yield arenarin C (4.5 mg).

Arenarin A (**1**): tan solid; mp 157−160 °C; HPLC t_R 20.7 min (70:30 CH_3OH−H_2O); UV λ_{max} (CH_3OH) 227 (ϵ 13 000), 318 (ϵ 11 000); IR (CH_2Cl_2) 3529, 2929, 1472, 1294 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.40 (s, 4-OH), 9.33 (s, 4″-OH), 9.12 (s, 5′-OH), 7.49 (s, H-6), 7.13 (d, J = 8.4 Hz, H-2″/6″), 7.06 (s, H-6), 7.04 (s, H-3), 6.77 (d, J = 8.4 Hz, H-3″/5″), 5.51 (m, H-8), 4.62 (br d, J = 6.6 Hz, H_2-7), 3.78 (s, 3′-OCH_3), 1.75 (s, H_3-10), 1.74 (s, H_3-11); ^{1}H,^{13}C, and HMBC NMR data, Table 1; NOESY data ($CDCl_3$; H-# ↔ H-#) H-3 ↔ 5′-OCH_3, H-6 ↔ H-6′, H-6 ↔ H_2-7, H-6 ↔ H-8, H_2-7 ↔ H-6′, H_2-7 ↔ H_3-11, H-8 ↔ H_3-10, H-2″ ↔ 5′-OCH_3; EIMS (70 eV) 406 (M^+, rel int 0.7), 350 (10), 338 (100), 305 (11), 277 (7), 237 (3), 221 (3), 165 (8), 138 (4), 105 (2); HRFABMS (DTT/DTE matrix) obsd 407.1499 $(M + H)^+$, calcd for $C_{24}H_{22}O_6$ + H 407.1495.

Arenarin B (**2**): tan solid; mp 109−112 °C; HPLC t_R 18.6 min (67:23 CH_3OH−H_2O); $[\alpha]_D$ +197° (c = 0.0004 g/mL, $CHCl_3$); UV λ_{max} (CH_3OH) 218 (ϵ 24 000), 293 (ϵ 18 000); IR (CH_2Cl_2) 3539, 2927, 1661, 1511, 1462, 1283 cm^{-1}; ^{1}H NMR (DMSO- d_6) δ 9.62 (s, 4′-OH), 8.91 (s, 6-OH), 7.46 (s, H-5), 7.10 (d, J = 8.4 Hz, H-2′/6′), 6.87 (s, H-8), 6.78 (d, J = 8.4 Hz, H-3′/5′), 5.67 (m, H-15), 4.88 (t, J = 5.4 Hz, 17-OH), 4.64 (dd, J = 12.0, 6.6 Hz, H-14), 4.60 (dd, J = 12.0, 6.6 Hz, H-14), 4.57 (s, H-12), 4.43 (s, H-12), 3.84 (br d, J = 3.6 Hz, H_2-17), 3.83 (s, 2-OCH_3), 3.60 (br t, J = 3.9 Hz, H-10), 2.89 (br d, J = 4.8 Hz, H_2-9), 1.654 (s, H_3-13), 1.649 (s, H_3-18); ^{1}H, ^{13}C, NMR data, Table 2; HMBC data, Table 2; HRFABMS (thioglycerol matrix) obsd 489.1916 $(M + H)^+$, calcd for $C_{29}H_{28}O_7$ + H 489.1913.

Arenarin C (**3**): tan solid; mp 107−110 °C; HPLC t_R 17.2 min (72:28 CH_3OH−H_2O); $[\alpha]_D$ −185° (c = 0.0025 g/mL, CH_3OH); UV λ_{max} (CH_3OH) 214 (ϵ 26 000), 290 (ϵ 18 000); IR (CH_2Cl_2) 3587, 2938, 1653, 1559, 1512 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.66 (s, 4′-OH), 7.59 (s, H-5), 7.10 (d, J = 8.4 Hz, H-2′/6′), 6.96 (s, H-8), 6.78 (d, J = 8.4 Hz, H-3′/5′), 5.66 (m, H-15), 4.90 (t, J = 5.7 Hz, 17-OH), 4.65 (dd, J = 11.7, 6.3 Hz, H-14), 4.60 (dd, J = 11.7, 6.3 Hz, H-14), 4.57 (s, H-12), 4.42 (s, H-12), 3.85 (br d, J = 4.2 Hz, H_2-17), 3.83 (s, 2-OCH_3), 3.76 (s, 6-OCH_3), 3.63 (br s, H-10), 2.94 (br s, H_2-9), 1.66 (s, H_3-13), 1.65 (s, H_3-18); ^{1}H and ^{13}C NMR data, Table 2; NOESY data (DMSO-d_6; H-# ↔ H-#) H-5 ↔ 6-OCH_3, H-8 ↔ H_2-9, H-8 ↔ H_2-14, H_2-14 ↔ H_3-18, H_2-17 ↔ H_3-18; HRFABMS (DTT/DTE matrix) obsd 503.2052 $(M + H)^+$, calcd for $C_{30}H_{30}O_7$ + H 503.2070.

Acknowledgment. We thank the National Science Foundation (CHE-9211252 and CHE-9708316) and Biotechnology Research and Development Corporation (no. 24-1-078) for financial support. We also thank the National Cancer Institute for cytotoxicity assay results.

Supporting Information Available: ^{1}H NMR and ^{13}C NMR spectra for compounds **1**−**3** (6 pages). Ordering information is given on any current masthead page.

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NP980001T