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Sequoiamonascins A−D: Novel Anticancer Metabolites Isolated from a Redwood Endophyte

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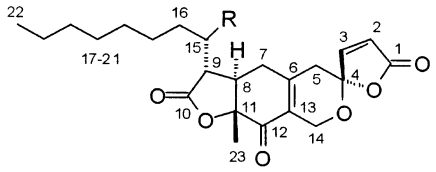
Abstract: *Aspergillus parasiticus*, a fungal isolate from the bark of a redwood tree (*Sequoia sempervirens*), has been shown to produce the antitumor metabolites sequoiatones A and B and more recently the sequoiatones C−F. We have also isolated another series of compounds with a new carbon skeleton, the sequoiamonascins. The structures of sequoiamonascins A−D were deduced by interpretation of their spectral data and that of some reaction products. The sequoiamonascins were isolated by brine shrimp lethality-guided fractionation and were submitted to the NCI for anticancer evaluation.

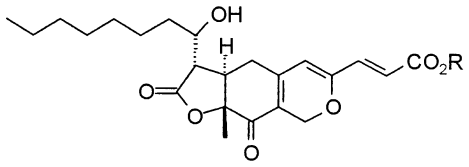
Investigating the secondary metabolites of microorganisms isolated from unusual or specialized ecological niches may increase the chance of finding novel, bioactive compounds. For the past 11 years, our laboratory has been investigating the endosymbiotic microbes of conifers that have received little attention as sources of bioactive compounds. Plant endosymbionts are generally nonpathogenic in nature but may produce compounds that enable them to survive in the competitive world of plant interstitial space. Some of these compounds could have useful medicinal or agrochemical applications.

An *Aspergillus parasiticus* isolate from the inner bark of a coast redwood tree (*Sequoia sempervirens*) has proven a prolific source of novel bioactive metabolites. Sequoiatones A and B,[1] and more recently sequoiatones C−F,[2] have been previously described. They were characterized by spectroscopic methods, and their absolute stereochemistry was established by X-ray crystallography. Brine shrimp lethality was used as an isolation guide for the sequoiatones. They showed moderate activity in the NCI primary in vitro human tumor cell line screen.[1] We are reporting the isolation and characterization of compounds with a new skeletal type, sequoiamonascins A−D, that were also isolated using brine shrimp lethality as a guide.

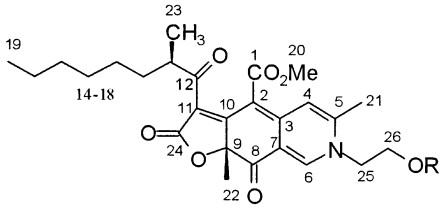
Sequoiamonacins A−C (**1**, **4,** and **5)** are isomeric compounds, with parent ions of *m*/*z* 418 in EIMS and molecular formulas of $C_{23}H_{30}O_7$, requiring nine degrees of unsaturation. The NMR spectral data for the sequoiamonascins was similar to that of the sequoiatones, but key disparities indicated a unique carbon framework.



1 R=OH
2 R=OAc
3 R=O
4 C4 epimer of 1



5 R=H
6 R=CH$_3$



7 R=H
8 R=Ac

The ^{13}C NMR data of sequoiamonascin A (**1**) (Table 1) showed a ketone carbon (δ 190.7), two ester carbons (δ 174.7 and 169.5), a disubstituted double bond (δ 124.4 and 152.6), and a tetrasustituted double bond (δ 146.8 and 128.2). Four rings accommodated the remaining sites of unsaturation. The IR spectrum displayed intense carbonyl absorption at 1775 cm^{-1} with a shoulder at 1755 and a second sharp peak at 1704 cm^{-1}. The UV spectrum, with a λ_{max} at 238 nm, indicated a conjugated dienyl system. These data and the ^{1}H−^{1}H COSY and HMBC NMR spectra of **1** and its derivatives were used to derive the structure of sequoiamonascin A.

The disubstituted double bond appeared as mutually coupled doublets at δ 7.23 and 6.20 (J = 5.7 Hz), which suggested a cis-α,β-unsaturated carbonyl system. The small coupling of these protons, together with the IR absorption at 1755 cm^{-1}, indicated an α,β-unsaturated γ-lactone.[3] In the HMBC spectrum, these olefinic protons showed long-range correlations to the carbonyl carbon at δ 169.5 (C-1) and to a ketal carbon at δ 104.0 (C-4). The ketal carbon showed further correlations to the

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(1) Stierle, A. A.; Stierle, D. B.; Bugni, T. *J. Org. Chem*. **1999**, *64*, 5479−5484.

(2) Stierle, A. A.; Stierle, D. B.; Bugni, T. *J. Nat Prod*. **2001**, *64*, 1350−1353.

(3) *Applications of Nuclear Magnetic Resonance in Organic Chemistry*; Jackman, L. M., Sternhell, S., Eds.; International Series of Monographs in Organic Chemistry; Pergammon Press: Oxford, 1969; Vol. 5, p 303.

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TABLE 1. NMR Data for Sequoiamonascin A (1)

	^{13}C	^{1}H	^{1}H COSY	HMBC
1	169.5s			
2	124.4d	6.20 (d, J = 5.7)	H-3	C-1, C-4
3	152.6d	7.23 (d, J = 5.7)	H-2	C-1, C-4
4	104.0s			
5α	36.2t	2.27 (bd, J = 18.8)	H-5β, H-14β	C-4, C-7
5β		2.87 (bd, J = 18.8)	H-5α, H-14α, H-14β	
6	146.8s			
7α	32.3t	2.59 (bd, J = 17.6)	H-7β, H-8, H-14α	C-5, C-6, C-9, C-13
7β		2.47 (m)	H-7α, H-8, H-14α, H-14β	
8	48.1d	2.80 (m)	H-7α, H-7β, H-9	C-7, C-9, C-10
9	43.2d	2.80 (m)	H-8, H-15	C-7, C-8, C-10
10	174.7s			
11	82.7s			
12	190.7s			
13	128.2s			
14α	61.2t	4.78 (bd, J = 16.2)	H-5β, H-7α, H-7β, H-14β	C-4, C-6, C-13
14β		4.47 (bd, J = 16.2)	H-5α, H-5β, H-7β, H-14α	
15	70.0d	3.83 (m)	H-9, H-16	
16	34.6t	1.69 (2H, m)	H-15, H-17	
17[a]	25.5t	1.26 (bs)		
18[a]	29.2t			
19[a]	29.4t			
20[a]	31.7t			
21[a]	22.6t		H-22	
22	14.0q	0.84 (3H, t, J = 7.0)	H-21	
23	16.8q	1.45 (3H, s)		C-11, C-12

[a] The carbon chemical shifts C-17 through C-21 were assigned by calculation. The J values are given in Hz.

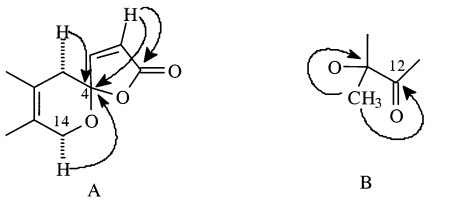

FIGURE 1. HMBC partial structures for sequoiamonacin A.

methylene protons on carbons C-5 and C-14 (Figure 1, A). These methylene protons also showed long-range correlations to another methylene carbon (C-7) at δ 32.3 ppm. Four bond correlations are not unprecedented in HMBC spectra.[1]

The C-7 methylene protons (δ 2.59 and 2.47) were spin-coupled to a methine proton (δ 2.80). Unfortunately, both H-8 and H-9 absorbed at δ 2.80 and could not be resolved with several different NMR solvents. One of them was also spin-coupled to methine proton H-15 (δ 3.83), which was coupled to a terminal *n*-heptyl chain, but it was not possible to resolve these protons in the natural product. Oxidation of sequoiamonacin A with PCC yielded a single product, diketone **3**, in which these ^{1}H NMR resonances could be assigned. The C-7 methylene protons (δ 2.59 and 2.36) were coupled to a ddd at δ 3.24 that was coupled to a sharp doublet at δ 3.66. This defined the C7−C9 fragment of sequoiamonacin A. The ^{1}H NMR spectrum of **1** also showed a methyl singlet at δ 1.45 that had

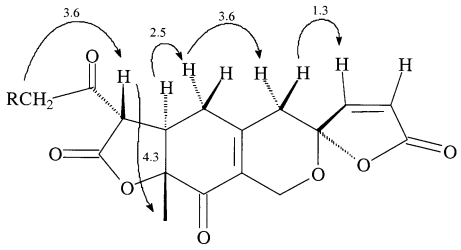

FIGURE 2. Relevant NOE interactions for diketone **3**.

HMBC correlations to a quaternary carbon at δ 82.7 (C-11) and to the ketone C-12 (Figure 1B).

Additional information was obtained from the acetylation product of **1**. Sequoiamonascin A formed a monoacetate **2** when treated with Ac_2O/Py. The ^{1}H NMR spectrum of **2** showed the expected downfield shift of the secondary alcohol but still had several overlapping signals in the δ 2.5 region of the ^{1}H NMR spectrum. The carbon framework that these data described is similar to the monascins, a family of compounds isolated from *Monascus* sp.[4] and the pitholides isolated from a *Pithomyces* sp.[5] Unlike the pitholides, however, the sequoiamonascins showed significant brine shrimp lethality.

The relative stereochemistry of sequoiamonascin A was established by NOE studies on diketone **3** (Figure 2). The ring protons could be assigned unequivocally by consideration of coupling constants and NOE interactions. Attempts to establish the absolute stereochemistry at C-15 by the Moser derivatization yielded ambiguous results.

Although the proton and carbon spectra of sequoiamonascin A (**1**) and B (**4**) were generally similar, they differed significantly in the area around the ketal carbon, C-4. NOE studies confirmed that they were C-4 epimers.

Sequoiamonascin C (**5**) contained one additional double bond and one less ring than **1** and **4**. The UV spectrum gave a λ_{max} at 386 nm that indicated a more conjugated system than the other two sequoiamonascins. The protons on the disubstituted double bond were shifted slightly but now had the vicinal coupling (J = 15.4 Hz) of a *trans*-alkene. Sequoiamonascin C reacted easily with diazomethane to give a methyl ester. These data were accommodated by structure **5**. When sequoiamonascin A was treated with *p*-toluenesulfonic acid in boiling toluene, a complex mixture was obtained. Reversed-phase HPLC of the mixture yielded sequoiamonascins A−C.

Sequoiamonascin D was isolated as a dark red oil. EIMS of sequoiamonascin D (**7**) gave a parent ion at *m*/*z* 471. HRMS of the parent ion of the monoacetate derivative **8** (*m*/*z* 513.2361) gave a molecular formula of $C_{26}H_{33}NO_7$ for sequoiamonascin D. This formula required 11 units of unsaturation. The UV absorption at λ_{max} 481 (log ϵ 3.54) indicated a greater degree of conjugation than the other compounds. The ^{13}C NMR showed 26 carbons with two ketone carbons (δ 201.9 and 192.9), two ester carbons (δ 171.6 and 166.7), and eight olefinic carbons. The remaining degrees of unsaturation required three rings. The EIMS showed the facile loss of *m*/*z* 113 typical

(4) Fielding, B. C.; Holker, J. S.; Jones, D. F.; Powell, D. G.; Richmond, K. W.; Robertson, A.; Whalley, W. B. *J. Chem. Soc.* **1961**, 4579−4589.

(5) Wang, G. Y.; Borgeson, B.; Crews, P. *Tetrahedron Lett.* **1997**, *38*, 8449−8452.

of the *sec*-octyl side chain found in the sequoiatones.[1] The HMBC spectrum showed long-range correlations of the ketone carbon C-12 (δ 201.9) to the methyl protons of C-23 (δ 1.08). Again, this was consistent with the sequoiatone side chain.

Several nitrogen-containing monascins are known.[6] All of these compounds are red oils, and each has a side chain attached to the nitrogen between the C-5 and C-6 positions. Sequoiamonascin D had a side chain donated from ethanolamine as demonstrated by the ^{1}H−^{1}H COSY-coupled methylene triplets at δ 4.18 and 3.93. Acetate **8** had a subsequent downfield shift of one set of these methylene protons. The main cyclic framework of **7** fit nicely into the nitrogen-containing monascin skeleton with an additional carbon, ester C-1, attached to C-2. This explained the upfield shift of C-2 and was consistent with the carboxylation pattern seen in both the sequoiatone and the pitholide skeletons. Sequoiamonascin D was a hybrid of a monascin cyclic structure with the side chain and carboxylation pattern found in the sequoiatone skeletons.

Brine shrimp lethality guided isolation of the sequoiamonascins. LD_{50}'s for these compounds ranged from 600 to 300 μM. These compounds were sent to the Development Therapeutic Program (DTP), Division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute (NCI), for evaluation as anticancer agents.[7] The DTP uses a one-dose preliminary anticancer assay against three cancer cell lines: MCF7 (breast), NCI-H460 (lung), and SF-268 (CNS). Results are reported as the percent of growth of the treated cells when compared to the untreated control cells. Compounds which reduce cancer cell growth to ≤32% are further evaluated in the full panel of 60 cell lines over a 5-log dose range. Only compounds **1** and **4** were active in the three-cell line screen. In the 60-cell line assay, compound **1** had a

compd	[M]	MCF7[a]	NCI-H460[a]	SF-268[a]
sequoiamonascin A (**1**)	1.00E-4	1	1	2
sequoiamonascin B (**4**)	1.00E-4	19	4	15

[a] Percent cell growth compared to untreated cells.

median log GI_{50} of −5.00, below the potency threshold established by NCI to warrant further study. In this assay, **1** showed selective activity toward all six leukemia cell lines, one breast cancer cell line, and two melanoma cell lines, with median values approaching −6.00. The C-4 epimer compound **4** had a median log GI_{50} of −4.83 and showed some selectivity toward leukemia cell line SR. Both sequoiamonascins A and B were generally more potent than the sequoiatones,[1] although sequoiatone B exhibited a $\log_{10}$ GI_{50} of <−7.00 against certain renal cell lines.

Experimental Section

Collection, Extraction, and Isolation Procedures. The collection, fungal isolation, and bioassay have been described previously.[1]

Fungus RDWD1-2 was identified as *Aspergillus parasiticus* by Microbial Identification, Inc. The $CHCl_3$ extract of the RDWD1-2 pilot culture showed little antimicrobial activity but good brine shrimp lethality. The fungus was grown in 54 × 300 mL DIFCO mycological broth cultures in 1 L Erlenmeyer flasks for 21 days, still. At harvest time, the culture was filtered through cheesecloth, and the mycelial mat and the filtrate were handled separately.

The mycelial mat was pulverized in a Waring blender and soaked in MeOH (2 L) overnight. It was filtered through Whatman filter paper and then extracted (2 L MeOH) and filtered two more times. The mat was air-dried (98.54 g), and the organic extract was reduced in vacuo and then partitioned between H_2O (1 L) and $CHCl_3$ (1 L). The $CHCl_3$ partition was reduced in vacuo to give an oil (9.80 g) that was active in the brine shrimp lethality bioassay. All subsequent procedures were guided by this bioassay. It was fractionated by LH-20 column chromatography ($CHCl_3$/MeOH, 1:1) to give several fractions. Fraction D was further resolved by flash silica gel chromatography and HPLC on silica gel with IPA/hexane mixtures to give **1** (85 mg, 0.87% $CHCl_3$ partition), **4** (175 mg, 1.8% $CHCl_3$ partition), **5** (34 mg, 0.35% $CHCl_3$ partition), and **7** (15 mg, 0.15% $CHCl_3$ partition).

Sequoiamonascin A (1): yellow solid; mp 52.5−53.5 °C; $[\alpha]^{25}_D$ +6.4 (*c* 0.17, MeOH); UV (MeOH) λ_{max} 208 (log ϵ 3.90), 238 (log ϵ 3.94); IR (neat) ν_{max} 3500, 2910, 1775, 1755, 1704, 1358, 1232, 1031 cm^{-1}; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR, see Table 1; EIMS 418(8), 319(8), 290(32), 192(100), 106(40), 94(48), 43(81); HREIMS *m/z* 400.1883 ($M^+ - H_2O$) ($M^+ - H_2O$ calcd for $C_{23}H_{28}O_6$, 400.1886).

Acetylation of Compound 1. Compound **1** (4.0 mg) was dissolved in 300 μL of pyridine and 300 μL of acetic anhydride and stirred for 24 h. The solvents were removed in vacuo and the residue was chromatographed on Si gel to give 3.2 mg of acetate **2**: ^{1}H NMR ($CDCl_3$) δ 7.21 (1H, d, J = 5.5 Hz), 6.22 (1H, d, J = 5.5 Hz), 5.19 (1H, dt, J = 9.5, 3.3 Hz), 4.78 (1H, bd, J = 16.1 Hz), 4.48 (1H, bdd, J = 16.1, 2.8 Hz), 2.94 (1H, dd, J = 13.2, 1.6 Hz), 2.87 (1H, bd, J = 18.7 Hz), 2.61 (2H, m), 2.39 (1H, m), 2.28 (1H, bd, J = 18.7 Hz), 2.06 (3H, s), 1.97 (2H, m), 1.45 (3H, s), 1.26 (bs), 0.86 (1H, t, J = 7.0 Hz); HREIMS *m/z* 460.2085 (M^+) (M^+ calcd for $C_{25}H_{32}O_8$ 460.2097).

Oxidation of Compound 1. Compound **1** (5.0 mg) was dissolved in CH_2Cl_2, and pyridinium chlorochromate (5.0 mg) was added. The mixture was stirred for 2 h, the solvent was then removed, and the diketone was chromatographed on Si gel to give 2.5 mg of diketone **3**: ^{1}H NMR ($CDCl_3$) δ 7.21 (1H, d, J = 5.5 Hz), 6.22 (1H, d, J = 5.5 Hz), 4.78 (1H, bdd, J = 16.1, 1.6 Hz), 4.48 (1H, bdd, J = 16.1, 2.8 Hz), 3.66 (1H, d, J = 12.8 Hz), 3.24 (1H, ddd, J = 15.8, 12.8, 4.4 Hz), 3.03 (1H, dt, J = 18.0, 7.7 Hz), 2.86 (1H, bd, J = 19.0 Hz), 2.60 (1H, dt, J = 18.0, 7.2 Hz), 2.59 (1H, m), 2.36 (1H, bd), 2.26 (1H, bd, J = 19.0 Hz), 1.49 (3H, s), 1.26 (bs), 0.86 (3H, t, J = 7.0 Hz).

Acid Isomerization of Compound 1. Compound **1** (5 mg) was dissolved in toluene, and *p*-TsOH (0.5 mg) was added. The mixture was heated to reflux for 2 h and cooled and the solvent removed. The residue was chromatographed on RPHPLC to give **1** (0.2 mg) and the two isomeric sequoiamonascins B (**4**) (0.6 mg) and C (**5**) (0.4 mg).

Sequoiamonascin B (4): yellow solid; mp 177.8−178.0 °C; $[\alpha]^{25}_D$ +55.9 (*c* 0.13, MeOH); UV (MeOH) λ_{max} 204 (log ϵ 4.08), 240 (log ϵ 4.04); IR (neat) ν_{max} 3510, 2010, 1707, 1700, 1075, 910 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.20 (1H, d, J = 5.5 Hz, H-3), 6.23 (1H, d, J = 5.5 Hz, H-2), 4.66 (1H, bd, J = 16.1 Hz, H14α), 4.52 (1H, bd J = 16.1 Hz, H-14β), 3.88 (1H, m, H-15), 2.85 (1H, bd, J = 18.8 Hz, H-5α), 2.79 (2H, m, H-8 and H-9), 2.55 (2H, m, H-7α and H-7β), 2.31 (1H, bd J = 18.8 Hz, H-5β), 1.59 (2H, m, H-16), 1.44 (3H, s, H-23), 1.26 (bs, H-17-H-21), 0.85 (3H, t, J = 6.6 Hz, H-23); ^{13}C NMR ($CDCl_3$) δ 191.7 (s, C-12), 174.6 (s, C-10), 169.6 (s, C-1), 152.8 (s, C-3), 147.9 (s, C-6), 127.4 (s, C-13), 124.0 (s, C-2), 104.1 (s, C-4), 82.8 (s, C-11), 69.4 (d, C-15), 61.3 (t, C-14), 48.0 (d, C-8), 42.9 (d, C-9), 36.2 (t, C-5), 33.9 (t, C-16), 32.6 (t, C-7), 31.6 (t, C-20), 29.2 (t, C-18), 29.0 (t, C-19), 25.5 (t, C-17), 22.3 (t, C-21), 16.5 (q, C-23), 13.9 (q, C-22); EIMS 418 (2), 400 (3), 290 (45), 272 (35), 192 (90), 106 (100), 94 (80); HREIMS *m/z* 418.1976 (M^+, calcd for $C_{23}H_{30}O_7$ 418.1991).

(6) Sweeny, J. G.; Estrada-Valdes, M. C.; Iacobucci, G. A.; Sato, H.; Sakamura, S. *J. Agric. Food Chem.* **1981**, *29*, 1189−1193.
(7) Boyd, M.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91−109.

Sequoiamonascin C (5): yellow solid; mp 191.6−192.5 °C; $[\alpha]^{25}_D$ −0.9 (*c* 0.17, MeOH); UV (MeOH) λ_{max} 242 (log ϵ 3.93), 386 (log ϵ 3.87); IR (neat) ν_{max} 3500, 2910, 1775, 1760, 1660, 1530, 970 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.05 (1H, d, J = 15.4 Hz, H-3), 6.42 (1H, d, J = 15.4 Hz, H-2), 5.67 (1H, s, H-5), 5.03 (1H, bd, J = 13.2 Hz, H-14α), 4.86 (1H, bd J = 13.2 Hz, H-14*β*), 3.84 (1H, m, H-15), 2.75 (2H, m, H-8 and H-9), 2.68 (1H, bd, J = 17.6 Hz, H-7α), 2.59 (1H, m, H-7*β*), 1.64 (2H, m, H-16), 1.44 (3H, s, H-23), 1.27 (bs, H-17-H21), 0.87 (3H, t, J = 7.0 Hz, H-22); ^{13}C NMR ($CDCl_3$) δ 190.4 (s, C-12), 175.2 (s, C-10), 169.8 (s, C-1), 156.6 (s, C-4), 147.9 (s, C-6), 137.5 (d, C-3), 122.3 (d, C-2), 118.3 (s, C-13), 110.9 (d, C-5), 83.2 (s, C-11), 70.3 (d, C-15), 63.9 (t, C-14), 48.3 (d, C-8), 43.6 (d, C-9), 34.7 (t, C-16), 31.8 (t, C-20), 30.2 (t, C-7), 29.4 (t, C-19), 29.2 (t, C-18), 25.4 (t, C-17), 22.6 (t, C-21), 17.2 (q, C-23), 14.1 (q, C-22); EIMS 418 (15), 391 (3), 290 (4), 192 (100), 164 (10), 106 (15); HREIMS *m*/*z* 418.1992 (M^+, calcd for $C_{23}H_{30}O_7$ 418.1991).

Methylation of Compound 5. Compound **5** (2 mg) was dissolved in Et_2O (500 μL) and MeOH (50 μL). A solution of diazomethane in Et_2O was added dropwise until the yellow color persisted. The solvents were evaporated to give methyl ester **6** (2 mg): ^1H NMR ($CDCl_3$) δ 7.02 (1H, d, J = 15.4 Hz), 6.43 (1H, d, J = 15.4 Hz), 5.64 (1H, s), 5.03 (1H, bd, J = 13.2 Hz), 4.86 (1H, bd J = 13.2 Hz), 3.84 (1H, m), 3.78 (3H, s), 2.75 (2H, m), 2.68 (1H, bd, J = 17.6 Hz), 2.59 (1H, m), 1.64 (2H, m), 1.44 (3H, s), 1.27 (bs), 0.87 (3H, t, J = 7.0 Hz).

Sequoiamonascin D (7): red oil; $[\alpha]^{25}_D$ +1.7 (*c* 0.09, MeOH); UV (MeOH) λ_{max} 202 (log ϵ 3.94), 260 (log ϵ 3.74), 402 (log ϵ 3.50), 481 (log ϵ 3.54); IR ($CHCl_3$) ν_{max} 3400, 3000, 1730, 1670, 1620, 1505, 1204, 1000, 740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.89 (1H, bs, H-6), 7.71 (1H, bs, H-4), 4.18 (2H, H-26), 3.93 (2H, H-25), 3.54 (3H, s, H-20), 2.41 (3H, bs, H-21), 1.65 (3H, s, H-22), 1.21 (bs, H-14-H-18), 1.08 (3H, d, J = 7.0 Hz), 0.82 (3H, t, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 201.9 (s, C-12), 192.9 (s, C-8), 171.6 (s, C-24), 170.2 (s, C-5), 166.7 (s, C-1), 148.6 (s, C-3), 147.4 (s, C-10), 143.0 (d, C-6), 117.1 (d, C-4), 115.5 (s, C-7), 110.7 (s, C-11), 95.8 (s, C-2), 86.1 (s, C-9), 60.7 (t, C-26), 55.8 (t, C-25); 50.7 (q, C-20), 43.0 (d, C-13), 31.8 (t, c-14), 31.6 (t, C-17), 29.5 (t, C-16), 27.5 (q, C-22), 27.2 (t, C-15), 22.6 (t, C-18), 20.1 (q, C-21), 17.2 (q, C-23), 14.1 (q, C-19); EIMS *m*/*z* 471 (5), 428 (5), 358 (100), 340 (15), 290 (15), 271 (18), 107 (70), 55 (98).

Acetylation of Compound 7. Compound **7** (2.0 mg) was dissolved in 200 μL of pyridine and 200 μL of acetic anhydride and stirred for 24 h. The solvents were removed in vacuo and the residue was chromatographed on Si gel to give 1.8 mg of acetate **8**: ^1H NMR ($CDCl_3$) δ 7.82 (1H, bs), 7.23 (1H, bs), 4.35 (2H), 4.12 (2H), 3.60 (3H, s), 2.41 (3H, bs), 2.07 (3H, s), 1.65 (3H, s), 1.21 (bs), 1.08 (3H, d, J = 7.0 Hz), 0.82 (3H, t, J = 7.0 Hz); EIMS *m*/*z* 513 (14), 470 (6), 400 (98), 373 (10), 332 (8), 87 (100); HREIMS *m*/*z* 513.2361 (M^+, calcd for $C_{28}H_{35}NO_8$ 513.2363).

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Supporting Information Available: ^1H NMR and ^{13}C NMR spectra for **1**, **4**, **5**, and **7**, COSY and HMBC spectra for **7**, and NCI cancer cell line assay for sequoiamonascins A (**1**) and B (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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