Allenyl and Alkynyl Phenyl Ethers from the Endolichenic Fungus *Neurospora terricola*

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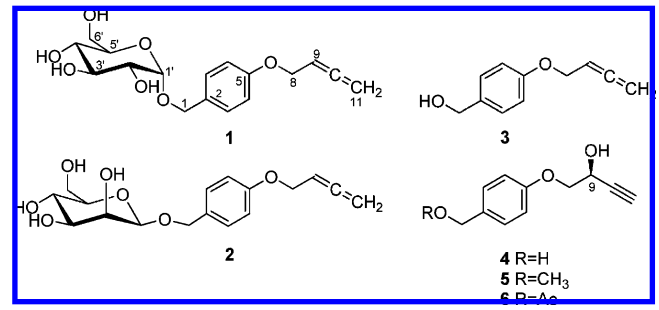
Terricollenes A−C (**1**−**3**), new allenyl phenyl ethers, and terricolynes (**4**−**6**), new alkynyl phenyl ethers, have been isolated from the crude extract of the endolichenic fungus *Neurospora terricola*. The structures of these compounds were elucidated primarily by NMR experiments. The absolute configurations of **1** and **2** were determined by reversed-phase HPLC analysis on the *o*-tolylthiocarbamates of the liberated sugars upon acid hydrolysis, whereas that of **4** was assigned using the modified Mosher method. Compound **1** showed modest cytotoxicity against the human tumor cell lines HeLa and MCF-7.

Endolichenic fungi are microorganisms living in the thalli of lichens that are analogous to the plant endophytic species inhabiting the intercellular spaces of the hosts.[1] Although numerous bioactive natural products have been isolated from their plant endophytic counterparts, only a limited number of secondary metabolites have been previously reported from the endolichenic fungi.[2,3] Examples include three heptaketides from an endolichenic *Corynespora* sp. inhabiting the cavern beard lichen *Usnea cavernosa*[2] and seven ambuic acid and one torreyanic acid derivative from a *Pestalotiopsis* sp. isolated from the lichen *Multiclavula* sp.[3] In an effort to identify new antitumor natural products from fungal species of unique niches, chemical investigations of fungi living with lichens were recently initiated in our laboratory.[3] In this study, the fungus *Neurospora terricola* (Sordariaceae) was isolated from the lichen *Everniastrum cirrhatum* (Fr.) Hale ex Sipman (Parmeliaceae) collected from Zixi Mountain, Yunnan Province, People's Republic of China. The fungus was grown in a solid-substrate fermentation culture. Its organic solvent extract showed cytotoxicity against two human tumor cell lines, HeLa and MCF-7. Fractionation of the extract led to the isolation of six new metabolites, which have been named terricollenes A−C (**1**−**3**), terricolyne (**4**), 1-*O*-methylterricolyne (**5**), and 1-*O*-acetylterricolyne (**6**). Compounds **1**−**3** are allenyl phenyl ethers, with **1** and **2** being glycosylated, whereas **4**−**6** are alkynyl phenyl ethers. Details of the isolation, structure elucidation, and cytotoxicity of these metabolites are presented herein.

Results and Discussion

Terricollene A (**1**) was isolated as a colorless oil. It was assigned the molecular formula $C_{17}H_{22}O_7$ (seven degrees of unsaturation) by HRESIMS (*m/z* 361.1262 $[M + Na]^+$; Δ −0.4 mmu). Analysis of the ^1H, ^{13}C, and HMQC NMR data (Table 1) revealed four exchangeable protons, three oxygenated sp^3 methylenes, five oxygenated methines, six aromatic carbons (four of which are protonated), and three sp^2 carbons (δ_C 76.6, 87.9, and 208.0) possibly due to the presence of an allenyl unit.[4] NMR resonances for the four aromatic protons were observed as two sets of doublets (8.5 Hz each) at δ_H 6.91 and 7.32, respectively, suggesting the presence of a *p*-substituted phenyl ring. The resonances for C-9 (δ_C 87.9), C-10 (δ_C 208.0), and C-11 (δ_C 76.6), together with an IR absorption at 1958 cm^{-1}, are indicative of an allenyl moiety.[4] This postulation was supported by HMBC correlations from H_2-11 to C-9 and C-10 and from H-9 to C-11. The $^1H-^1H$ COSY correlation between H_2-8 and H-9 and relevant HMBC cross-peaks led to the connection of C-8 to C-9, completing the buta-1,2-diene moiety (C-8−C-11). HMBC correlations from H_2-1 to C-2 and from H-3 and H-7 to C-1 defined the *p*-substituted benzyl unit, whereas that from H_2-8 to C-5 established the [4-(buta-2,3-dienyloxy)phenyl]methanol partial structure. The remaining resonances in **1** were attributed to a hexopyranose unit on the basis of its ^1H and ^{13}C NMR data (Table 1). The large *trans*-diaxial-type $^1H-^1H$ coupling constants observed between H-2′ and H-3′ (9.6 Hz), H-3′ and H-4′ (9.6 Hz), and H-4′ and H-5′ (9.0 Hz) indicated that they are all in axial orientations with respect to the pyranosyl residue, leading to assignment of a glucose (Glc) moiety. An HMBC correlation from the anomeric proton of Glc (H-1′; δ_H 4.84) to the oxygenated methylene carbon (C-1; δ_C 69.2) of the benzyl unit led to the connection of the Glc unit to the [4-(buta-2,3-dienyloxy)phenyl-]methanol moiety at C-1. On the basis of these data, the gross structure of terricollene A was established as **1**.



The Glc unit in **1** was connected to the [4-(buta-2,3-dienyloxy)phenyl]methanol moiety via an α-linkage on the basis of the chemical shift of the anomeric carbon (C-1′; δ_C 99.0), as well as the $^1H-^1H$ coupling constant (4.2 Hz) observed for the anomeric proton (H-1′). The absolute configuration of Glc in **1** was determined by RP HPLC analysis of its *o*-tolylthiocarbamate. Treatment of the liberated Glc with L-cysteine methyl ester, followed by *o*-tolylisothiocyanate, afforded a derivative matching that of the D-Glc by RP HPLC analysis in comparison with the D- and L-standards.[5,6] The absolute configuration assigned for Glc in **1** was also confirmed by comparison of the specific rotation of **1** ($[\alpha]^{25}_D$ +110.0) with that ($[\alpha]^{25}_D$ +121.5) reported for α-D-Glc.[7]

Terricollene B (**2**) was assigned the same molecular formula, $C_{17}H_{22}O_7$, as **1** on the basis of its HRESIMS (*m/z* 361.1255 $[M + Na]^+$; Δ +0.3 mmu). Although its NMR spectra revealed structural

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Table 1. NMR Data (500 MHz, acetone-d_6; 600 MHz, methanol-d_4) for Terricollenes A (**1**) and B (**2**)

	1				**2**	
position	δ_C[a], mult.	δ_H[b] (*J* in Hz)	δ_H[c] (*J* in Hz)	HMBC[a,b]	δ_C[a], mult.	δ_H[b] (*J* in Hz)
1	69.2, CH_2	4.44, d (12); 4.69, d (12)	4.43, d (12); 4.63, d (12)	2, 1′	70.4, CH_2	4.57, d (12); 4.80, d (12)
2	131.3, qC				131.2, qC	
3	130.3, CH	7.32, d (8.5)	7.27, d (9.0)	1, 4, 5, 6	130.4, CH	7.29, d (8.5)
4	115.4, CH	6.91, d (8.5)	6.83, d (9.0)	2, 5	115.4, CH	6.91, d (8.5)
5	159.0, qC				159.0, qC	
6	115.4, CH	6.91, d (8.5)	6.83, d (9.0)	2, 5	115.4, CH	6.91, d (8.5)
7	130.3, CH	7.32, d (8.5)	7.27, d (9.0)	1, 4, 5, 6	130.4, CH	7.29, d (8.5)
8	66.4, CH_2	4.58, dt (2.5, 7.0)	4.50, dt (2.4, 6.6)	5, 9, 10	66.3, CH_2	4.58, dt (2.5, 7.0)
9	87.9, CH	5.41, tt (7.0, 7.0)	5.32, tt (6.6, 6.6)	11	87.9, CH	5.42, tt (7.0, 7.0)
10	208.0, qC				210.1, qC	
11	76.6, CH_2	4.88, dt (2.5, 6.5)	4.80, dt (2.4, 6.6)	8, 9, 10	76.6, CH_2	4.88, dt (2.5, 6.5)
1′	99.0, CH	4.84, d (3.5)	4.80, d (4.2)	2′, 3′	100.1, CH	4.58, s
2′	73.5, CH	3.36, m	3.33, dd (4.2, 9.6)		71.8, CH	3.83, d (3.0)
3′	75.3, CH	3.66, m	3.60, dd (9.6, 9.6)	4′	75.3, CH	3.40, dd (3.0, 9.0)
4′	72.0, CH	3.34, m	3.24, dd (9.0, 9.6)	2′ (or 5′), 3′, 6′	69.3, CH	3.58, dd (9.0, 9.0)
5′	73.5, CH	3.64, m	3.55, ddd (2.4, 6.0, 9.0)		77.8, CH	3.21, ddd (3.0, 6.0, 9.0)
6′	62.9, CH_2	3.66, m; 3.79, m	3.61, dd (6.0, 12); 3.73, dd (2.4, 12)	5′	63.1, CH_2	3.67, dd (6.0, 12); 3.86, dd (3.0, 12)
OH-2′		3.48[e], s				
OH-3′		3.50[e], s				
OH-4′		3.97[e], s				
OH-6′		4.05[e], s				

[a] Recorded at 125 MHz in acetone-d_6. [b] Recorded at 500 MHz in acetone-d_6. [c] Recorded at 600 MHz in methanol-d_4. [d] HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon. [e] These assignments are interchangeable.

Table 2. NMR Data (500 MHz, acetone-d_6) for Compounds **4**−**6**

	4			**5**		6	
position	δ_C, mult.	δ_H (*J* in Hz)	HMBC[a]	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)
1	64.2, CH_2	4.54, s	2, 3, 7	61.3, CH_2	4.34, s	66.2, CH_2	5.01, s
2	135.9, qC			132.1, qC		130.0, qC	
3	128.9, CH	7.27, d (8.5)	1, 4, 5	130.0, CH	7.25, d (8.5)	130.8, CH	7.32, d (8.5)
4	115.2, CH	6.91, d (8.5)	2, 3, 5	115.3, CH	6.93, d (8.5)	115.4, CH	6.96, d (8.5)
5	158.7, qC			159.1, qC		159.6, qC	
6	115.2, CH	6.91, d (8.5)	2, 5, 7	115.3, CH	6.93, d (8.5)	115.4, CH	6.96, d (8.5)
7	128.9, CH	7.27, d (8.5)	1, 5, 6	130.0, CH	7.25, d (8.5)	130.8, CH	7.32, d (8.5)
8	72.7, CH_2	4.07, m	5, 9, 10	72.7, CH_2	4.07, m	72.7, CH_2	4.09, m
9	61.3, CH	4.68, s	8, 10, 11	61.3, CH	4.69, d (5.0)	61.3, CH	4.69, s
10	83.7, qC			83.7, qC		83.7, qC	
11	74.5, CH	2.93, s	8, 9	74.5, CH	2.93, d (2.5)	74.5, CH	2.93, d (2.0)
12				57.2, CH_3	3.27, s	170.8, qC	
13						20.8, CH_3	2.00, s
OH-1		4.02, s					
OH-9		4.78, s	8, 9, 10		4.78, d (5.0)		4.80, s

[a] HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon.

similarity to **1**, the resonances for the sugar unit were significantly different from those in **1**, suggesting that **2** could be an analogue of **1** with a different sugar moiety. Analysis of the $^1H-^1H$ coupling constants of **2** (Table 1) established the identity of the sugar moiety. The *trans*-diaxial arrangement for H-3′, H-4′, and H-5′ was deduced from the large coupling constants (9.0 Hz), whereas the *cis* relationship for H-2′ and H-3′ was evident on the basis of their small coupling constant (3.0 Hz). Therefore, the sugar moiety in **2** was identified as mannose (Man).

The Man unit in **2** was attached to the aglycone via a β-linkage on the basis of the near-zero coupling constant observed for the anomeric proton (H-1′) and was supported by NOESY data (Figure S21 in the Supporting Information). NOESY correlations of H-1′ with H-3′ and H-5′ and of H-3′ with H-5′ suggested an axial orientation for the anomeric proton. The D-configuration was assigned for Man by comparison of the specific rotation of **2** ($[\alpha]^{25}_D$ −16.0) with that ($[\alpha]^{25}_D$ −16.5) reported for β-D-Man[7] and was further confirmed by RP HPLC analysis of the *o*-tolylthiocarbamate of Man as described above.

Terricollene C (**3**) was assigned the molecular formula $C_{11}H_{12}O_2$ (six degrees of unsaturation) on the basis of its HREIMS (*m*/*z* 176.0839 $[M]^+$; Δ −0.2 mmu). The mass spectrum of **3** displayed $[M - 53]^+$ and $[M - 31]^+$ peaks characteristic of the fragments with loss of a buta-1,2-diene and a hydroxymethyl unit, respectively, from the molecular ion. Analysis of its ^1H and ^{13}C NMR spectroscopic data revealed structural features similar to those found in **1**, except that the signals for the sugar moiety were absent, suggesting that **3** was the aglycone of **2**. A literature search identified a synthetic intermediate for natural allenyl phenyl ethers possessing the same structure as **3**.[8,9] However, this is the first demonstration of the natural occurrence of compound **3**.

Terricolyne (**4**) gave a pseudomolecular ion $[M + Na]^+$ peak at *m*/*z* 215.0684 (Δ −0.5 mmu) by HRESIMS, consistent with a molecular formula of $C_{11}H_{12}O_3$ (six degrees of unsaturation). Analysis of the ^1H, ^{13}C, and HMQC NMR data of **4** (Table 2) revealed the same *p*-substituted 4-(hydroxymethyl)phenyl unit as found in **1**−**3**, but the resonances for the allenyl portion of **1**−**3** were significantly different from those for the subunit connected to C-8 in **4**. The signals for H-11 (δ_H 2.93) and C-10 (δ_C 83.7) and C-11 (δ_C 74.5) are indicative of an ethynyl moiety.[10] The C-8−C-9 (including OH-9) fragment was identified by $^1H-^1H$ COSY correlations, and C-9 was connected to C-10 on the basis of HMBC correlations from H-11 to C-9 and from H-9 to C-8, C-10, and C-11 to establish the C-8−C-11 partial structure. An HMBC cross-peak from H_2-8 to C-5 established the ether linkage between C-5 and C-8, thereby completing the gross structure of **4** as shown.

The absolute configuration of **4** was assigned using the modified Mosher method.[11] Treatment of **4** with (*S*)-MTPACl and (*R*)-

Table 3. Cytotoxicity of Compounds **1**−**6**

	IC_{50} (μM)	
compound	HeLa	MCF-7
1	53.3	59.2
2	>118.3	>118.3
3	85.2	113.6
4	>200.0	>200.0
5	92.6	115.7
6	>170.9	>170.9
5-fluorouracil	10.0	15.0

MTPACl afforded the (*R*)-MTPA (**4a**) and (*S*)-MTPA (**4b**) esters, respectively. The differences in chemical shift values ($\Delta\delta = \delta_S - \delta_R$) for the diastereomeric esters **4b** and **4a** were calculated to assign the 9*R* absolute configuration on the basis of the $\Delta\delta$ results summarized in Figure S22 (Supporting Information).

To verify that **4** is an authentic natural product, a portion of freeze-dried fermented rice substrate was extracted with distilled HPLC-grade EtOAc, and the resulting extract was analyzed by RP HPLC using distilled, HPLC-grade H_2O and MeCN as solvents. Compound **4** was identified on the HPLC chromatogram of the crude extract by comparison of its retention time with that of the pure compound, indicating that **4** is indeed a naturally occurring metabolite.

The elemental composition of 1-*O*-methylterricolyne (**5**) was established as $C_{12}H_{14}O_3$ (six degrees of unsaturation) by analysis of its HRESIMS data (*m*/*z* 229.0843 $[M + Na]^+$; Δ −0.8 mmu). The NMR spectra of **5** showed resonances nearly identical to those of **4**, except that the exchangeable proton at δ_H 4.02 (OH-1) was replaced by an *O*-methyl group (δ_H/δ_C 3.27/57.2) in the spectra of **5**, indicating that the C-1-OH was methylated. Therefore, the gross structure of 1-*O*-methylterricolyne was established as **5**, and its absolute configuration was deduced by analogy to **4**.

The molecular formula of 1-*O*-acetylterricolyne (**6**) was determined to be $C_{13}H_{14}O_4$ (seven degrees of unsaturation) on the basis of its HRESIMS (*m*/*z* 257.0786 $[M + Na]^+$; Δ −0.2 mmu). The extra 42 mass units, compared to that of **4**, suggested the presence of an acetyl group. Analysis of the ^1H and ^{13}C NMR spectroscopic data of **6** revealed structural similarity to those of **4**, except that the oxygenated methylene protons (H_2-1) were shifted downfield to δ_H 5.01 in **6**. In addition, NMR resonances corresponding to an acetyl group (δ_H 2.00; δ_C 20.8 and 170.8) were observed, indicating that the C-1 oxygen of **6** was acylated. The absolute configuration of **6** was also deduced by analogy to **4**.

Compounds **1**−**6** were evaluated for cytotoxicity against two human tumor cell lines, HeLa and MCF-7 (Table 3). Compounds **1**, **3**, and **5** showed modest cytotoxic activity against HeLa cells, with IC_{50} values ranging from 53.3 to 92.6 μM, whereas **1** also displayed activity against the MCF-7 cells, with an IC_{50} value of 59.2 μM (the positive control 5-fluorouracil showed IC_{50} values of 10.0 and 15.0 μM, respectively, against the two tumor cell lines). These metabolites were not further evaluated for their antitumor effects against any molecular targets due to their modest cytotoxic effects.

Compounds **1**−**3** possess the same *p*-(buta-2,3-dienyl ether)phenyl moiety as found in xyloallenolide A[4] and the eucalyptenes.[12] However, **1** and **2** differ significantly from the known metabolites by incorporating a Glc and a Man unit, respectively. Compounds **4**−**6** are new analogues of penipratynolene.[10] Compound **4** possesses a hydroxymethyl group at C-2, whereas **5** and **6** are the methylated and acylated products of **4**, respectively, compared to an acetyl group at C-2 in the known metabolite.

Over 150 natural products incorporating an allenyl moiety have been reported with enzyme inhibitory, cytotoxic, and antiviral activities.[13−15] Studies on structure−activity relationships suggested that the introduction of the allenyl moiety could generate or enhance cytotoxicity of these compounds.[8] Alkynes are important bioactive natural products showing cytotoxic, anti-inflammatory, and antiprotozoal effects[16−18] and are commonly used as the building blocks in synthesis of allenyl natural products.[19−22]

Compounds **1**−**6** are the first secondary metabolites to be reported from the endolichenic fungus *Neurospora* sp. and the third examples of natural products discovered from endolichenic fungal sources.[2,3]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and UV data were recorded on a Shimadzu Biospec-1601 spectrophotometer. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. ^1H and ^{13}C NMR data were acquired with Varian Mercury-500 and -600 spectrometers using solvent signals (acetone-d_6, δ_H 2.05/δ_C 29.8, 206.1; methanol-d_4, δ_H 3.35/δ_C 49.9) as references. The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS and HRESIMS data were recorded on a Mariner ESI-TOF spectrometer. EIMS and HREIMS data were recorded on a Micromass GCT-MS spectrometer.

Fungal Material. The culture of *N. terricola* (Sordariaceae) was isolated by one of the authors (L.G.) from samples of the lichen *E. cirrhatum* collected from Zixi Mountain, Yunnan Province, in November 2006. The fungus was identified by L.G. and assigned the accession number 63-9-3-2 in L.G.'s culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. Agar plugs were inoculated in 250 mL Erlenmeyer flasks, each containing 50 mL of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract), and the final pH of the media was adjusted to 6.5 before sterilization. Flask cultures were incubated at 25 °C on a rotary shaker at 170 rpm for five days. The fungal strain was cultured on slants of potato dextrose agar at 25 °C for 10 days. Fermentation was carried out in four 500 mL Fernbach flasks each containing 80 g of rice. Spore inoculum was prepared by suspension in sterile, distilled H_2O to give a final spore/cell suspension of 1×10^6/mL. Distilled H_2O (120 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 lb/in^2. for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at 25 °C for 40 days.

Extraction and Isolation. The fermented rice substrate was extracted repeatedly with EtOAc (3 × 600 mL), and the organic solvent was evaporated to dryness under vacuum to afford the crude extract (1.9 g), which was fractionated by silica gel VLC using petroleum ether−EtOAc gradient elution. The fractions eluted with 15% (100 mg) and 20% (80 mg) EtOAc were combined and separated again by Sephadex LH-20 column chromatography (CC) using 1:1 CH_2Cl_2−MeOH as eluents. The resulting subfraction (45 mg) was further separated by RP HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 43% MeOH in H_2O for 2 min, followed by 43−56% MeOH in H_2O for 40 min; 2 mL/min) to afford **5** (5.0 mg, t_R 24.8 min), **6** (6.0 mg, t_R 28.7 min), and **3** (5.0 mg, t_R 37.0 min). The fractions eluted with 25% (100 mg) and 30% (100 mg) EtOAc were combined and separated again by Sephadex LH-20 CC eluting with CH_2Cl_2−MeOH (1:1). Purification of the resulting subfractions (30 mg) by RP HPLC (35% MeOH in H_2O for 2 min, followed by 35−55% MeOH in H_2O for 40 min) afforded **4** (11.5 mg, t_R 14.5 min). The fractions eluted with 70% and 80% EtOAc were combined (170 mg) and fractionated by Sephadex LH-20 CC eluting with MeOH. Further purification of the resulting subfraction (10 mg) by RP HPLC (40% MeOH in H_2O for 2 min, followed by 40−55% MeOH in H_2O for 40 min) afforded **1** (1.5 mg, t_R 28.3 min) and **2** (1.0 mg, t_R 30.9 min).

Terricollene A (1): colorless oil; $[\alpha]^{25}_D$ +110.0 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 215 (4.04), 230 (4.09) nm; IR (neat) ν_{max} 3375 (br), 2928, 1958, 1611, 1585, 1512, 1460, 1412, 1380, 1243, 1033 cm^{-1}; ^1H, ^{13}C NMR and HMBC data see Table 1; HRESIMS *m*/*z* 361.1262 (calcd for $C_{17}H_{22}O_7Na$, 361.1258).

Terricollene B (2): white needles (acetone); mp 89−90 °C; $[\alpha]^{25}_D$ −16.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.24), 230 (4.22) nm; IR (neat) ν_{max} 3333 (br), 2936, 2879, 1957, 1612, 1585, 1459, 1383, 1252, 1077 cm^{-1}; ^1H and ^{13}C NMR data (acetone-d_6, 500 MHz) see Table 1; ^1H NMR (methanol-d_4, 600 MHz) δ 7.25 (2H, d, J = 8.4 Hz, H-3, H-7), 6.84 (2H, d, J = 8.4 Hz, H-4, H-6), 5.32 (1H, tt, J = 6.6, 6.6 Hz, H-9), 4.80 (2H, dt, J = 2.4, 6.6 Hz, H-11), 4.78 (1H, d, J = 12 Hz, H-1b), 4.55 (1H, d, J = 12 Hz, H-1a), 4.50 (2H, dt, J = 2.4, 6.6 Hz, H-8), 4.46 (1H, s, H-1′), 3.85 (1H, dd, J = 2.4, 12 Hz, H-6′b),

3.77 (1H, d, $J = 3.6$ Hz, H-2′), 3.68 (1H, dd, $J = 6.0$, 12 Hz, H-6′a), 3.50 (1H, dd, $J = 9.6$, 9.6 Hz, H-4′), 3.34 (1H, dd, $J = 3.6$, 9.6 Hz, H-3′), 3.13 (1H, ddd, $J = 2.4$, 6.0, 9.6 Hz, H-5′); HMBC data (acetone-d_6, 500 MHz) H_2-1 → C-2, 1′; H-3 → C-1, 4, 5, 6; H-4 → C-2, 5; H-6 → C-2, 5; H-7 → C-1, 4, 5, 6; H_2-8 → C-5, 9; H_2-11 → C-9; H-9 → C-11; H-1′ → C-1, 2′; H-2′ → C-3′, 4′; H-3′ → C-4′; H-4′ → C-3′, 5′, 6; H_2-6′ → C-4′; NOESY correlations (methanol-d_4, 600 MHz) H-1′ ↔ H-3′, H-5′; H-3′ ↔ H-5′; H-1a ↔ (H-3 or H-7); HRESIMS *m/z* 361.1255 (calcd for $C_{17}H_{22}O_7Na$, 361.1258).

Terricollene C (3): white powder; $[\alpha]^{25}_D$ +2.0 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 215 (3.63), 244 (3.67), 255(3.70) nm; IR (neat) ν_{max} 3359 (br), 2924, 2855, 1958, 1631, 1586, 1420, 1299, 1241, 1174, 1013 cm^{-1}; ^1H NMR data (500 MHz, acetone-d_6) δ 7.25 (2H, d, $J = 8.5$ Hz, H-3/H-7), 6.88 (2H, d, $J = 8.5$ Hz, H-4/H-6), 5.41 (1H, tt, $J = 7.0$, 7.0 Hz, H-9), 4.86 (2H, m, H-11), 4.56 (2H, m, H-8), 4.53 (2H, s, H_2-1), 3.99 (1H, s, OH-1); ^{13}C NMR data (acetone-d_6, 100 MHz) δ 210.1 (C, C-10), 158.4 (C, C-5), 135.7 (C, C-2), 128.8 (CH, C-3, C-7), 115.3 (CH, C-4, C-6), 87.9 (CH, C-9), 76.5 (CH_2, C-11), 66.4 (CH_2, C-8), 64.3 (CH_2, C-1); HREIMS *m/z* 176.0839 (calcd for $C_{11}H_{12}O_2$, 176.0837).

Terricolyne (4): white powder; $[\alpha]^{25}_D$ −3.0 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.59), 267 (3.58) nm, 277 (3.60) nm; IR (neat) ν_{max} 3357, 3286, 2928, 2877, 2119, 1612, 1513, 1423, 1248, 1177, 1044 cm^{-1}; ^1H, ^{13}C NMR and HMBC data see Table 2; HRESIMS *m/z* 215.0684 (calcd for $C_{17}H_{22}O_7Na$, 215.0679).

1-*O*-Methylterricolyne (5): white powder; $[\alpha]^{25}_D$ −2.0 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 224 (3.79) nm, 233 (3.78) nm, 265 (3.81) nm, 276 (3.81) nm; IR (neat) ν_{max} 3364, 3288, 2929, 2859, 2118, 1612, 1513, 1421, 1301, 1247, 1177, 1089 cm^{-1}; ^1H and ^{13}C NMR data see Table 2; HRESIMS *m/z* 229.0843 (calcd for $C_{17}H_{22}O_7Na$, 229.0835).

1-*O*-Acetylterricolyne (6): white powder; $[\alpha]^{25}_D$ −3.0 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 260 (3.74), 270 (3.74) nm; IR (neat) ν_{max} 3434, 3287, 2939, 2880, 2119, 1734, 1613, 1514, 1455, 1302, 1245, 1178, 1089 cm^{-1}; ^1H and ^{13}C NMR data see Table 2; HRESIMS *m/z* 257.0786 (calcd for $C_{17}H_{22}O_7Na$, 257.0784).

Sugar Identification.[5,6] Authentic samples of D-Glc and L-cysteine methyl ester hydrochloride (5.0 mg each) were dissolved in pyridine (1.0 mL) and heated at 60 °C, and then *o*-tolylisothiocyanate (0.2 mL) was added to the mixture and heated at 60 °C for 1 h. The derivatives of L-Glc, D-Man, and L-Man (5.0 mg each) standards were prepared following the same procedure as described for that of D-Glc.

A sample of **1** (0.5 mg) in 300 μL of acetone was added to 700 μL of 6 N HCl in a hydrolysis tube, and the mixture was heated at 100 °C for 24 h. The reaction was quenched with 3.0 mL of H_2O and extracted twice with 2.0 mL of $CHCl_3$ to remove the aglycone. After evaporation of the aqueous layer, 1.0 mg of L-cysteine methyl ester hydrochloride and 100 μL of pyridine were added, and the mixture was stirred at 60 °C for 1 h. *o*-Tolylisothiocyanate (100 μL) was added, and the solution was stirred at 60 °C for another 1 h. The reaction mixture was directly analyzed by RP HPLC (Kromasil 100-5 C_{18} column; 4.6 × 250 mm; 25% CH_3CN in H_2O with 0.2% TFA; 0.8 mL/min; 25 °C) detected at 250 nm. The resulting Glc derivative (t_R 20.07 min) coeluted with a derivatized D-Glc standard (t_R 20.01 min), but not with a derivatized L-Glc standard (t_R 18.53 min).

Similarly, a sample of **2** (0.4 mg) was used for derivatization and RP HPLC analysis. The resulting Man derivative (t_R 15.92 min) coeluted with a derivatized D-Man standard (t_R 15.94 min), but not with a derivatized L-Man standard (t_R 20.16 min).

Preparation of (*R*)-MTPA Ester (4a) and (*S*)-MTPA Ester (4b). A sample of **4** (2.0 mg, 0.010 mmol) was dissolved in CH_2Cl_2 (3.0 mL) in a 10 mL round-bottomed flask. DMAP (8.0 mg) and (*S*)-MTPACl (10.0 μL, 0.052 mmol) were quickly added, the flask was sealed, and the mixture was stirred at room temperature for 12 h. The mixture was evaporated to dryness and purified by RP HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 70% MeOH in H_2O for 30 min; 2 mL/min) to afford **3a** (1.5 mg): colorless oil; ^1H NMR (acetone-d_6, 600 MHz) δ 7.35 (2H, d, $J = 8.4$ Hz, H-3/H-7), 6.92 (2H, d, $J = 8.4$ Hz, H-4/H-6), 6.07 (1H, ddd, $J = 2.4$, 3.6, 7.8 Hz, H-9), 5.35 (1H, d, $J = 12$ Hz, H-1a), 5.31 (1H, d, $J = 12$ Hz, H-1b), 4.40 (1H, dd, $J = 3.6$, 11 Hz, H-8a), 4.27 (1H, dd, $J = 7.8$, 11 Hz, H-8b), 3.37 (1H, d, $J = 2.4$ Hz, H-11).

In a similar fashion, a sample of **4** (3.0 mg, 0.015 mmol), CH_2Cl_2 (3.0 mL), DMAP (8.0 mg), and (*R*)-MTPACl (5.0 μL, 0.026 mmol) were allowed to react in a 10 mL round-bottomed flask at room temperature for 12 h, and the reaction mixture was processed as described above for **4a** to afford **4b** (1.0 mg): colorless oil; ^1H NMR (acetone-d_6, 400 MHz) δ 7.38 (2H, d, $J = 8.4$ Hz, H-3/H-7), 7.01 (2H, d, $J = 8.4$ Hz, H-4/H-6), 6.04 (1H, ddd, $J = 2.4$, 3.6, 7.8 Hz, H-9), 5.37 (H, d, $J = 12$ Hz, H-1a), 5.33 (H, d, $J = 12$ Hz, H-1b), 4.45 (1H, dd, $J = 3.6$, 11 Hz, H-8a), 4.35 (1H, dd, $J = 7.8$, 11 Hz, H-8b), 3.31 (1H, d, $J = 2.4$ Hz, H-11).

MTT Assay.[23] The assay was run in triplicate. In 96-well plates, each well was plated with 10^4 cells. After cell attachment overnight, the medium was removed, and each well was treated with 50 μL of medium containing 0.2% DMSO or appropriate concentrations of test compounds (10 mg/mL as stock solution of a compound in DMSO and serial dilutions; the test compounds showed good solubility in DMSO and did not precipitate when added to the cells). Cells were treated at 37 °C for 4 h in a humidified incubator at 5% CO_2 first and then were allowed to grow for another 48 h after the medium was changed to fresh Dulbecco's modified Eagle medium. MTT (Sigma) was dissolved in serum-free medium or PBS at 0.5 mg/mL and sonicated briefly. In the dark, 50 μL of MTT/medium was added into each well after the medium was removed from wells, and incubated at 37 °C for 3 h. Upon removal of MTT/medium, 100 μL of DMSO was added to each well, and the plate was agitated at 60 rpm for 5 min to dissolve the precipitate. The assay plate was read at 540 nm using a microplate reader.

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Supporting Information Available: ^1H and ^{13}C NMR spectra of terricollenes A−C (**1**−**3**), terricolyne (**4**), 1-*O*-methylterricolyne (**5**), and 1-*O*-acetylterricolyne (**6**). This material is available free of charge via the Internet at http://pubs.acs.org.

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