DOI: 10.1002/ejoc.200900735

Trichocladinols A–C, Cytotoxic Metabolites from a *Cordyceps*-Colonizing Ascomycete *Trichocladium opacum*

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Keywords: Cytotoxicity / Fungal metabolites / Structure elucidation / Natural products / Configuration determination

Trichocladinols A–C (**1**–**3**), three new metabolites, and the known massarigenin A (**4**), have been isolated from cultures of a *Cordyceps*-colonizing ascomycete *Trichocladium opacum*. Their structures were elucidated by NMR spectroscopy and X-ray crystallography. The absolute configuration of **1** was assigned by using the modified Mosher method and that of **3** was determined by X-ray crystallographic analysis of its (*S*)-MTPA ester. Compounds **1**–**3** showed modest cytotoxic effects against the human tumor cell lines HeLa and MCF-7. Structurally, compounds **1** and **2** possess a previously undescribed 2,9-dioxatricyclo[5.2.1.$0^{3,8}$]dec-4-ene skeleton.

Introduction

Cordyceps sinensis (Berk.) Sacc. (Anamorph: *Hirsutella sinensis*),[1] is a unique black, blade-shaped fungus found primarily at high altitude on the Qinghai-Tibetan plateau parasitizing on dead caterpillars of the moth *Hepilus* spp. A combination of the fungus and the dead caterpillars is known as "Dong Chong Xia Cao" (winter-worm, summer-grass) and has been widely used as a tonic and/or medicine for hundreds of years in the Orient.[2] Even though many medical benefits, including antitumor activity, have been reported for this fungus,[3] whether the active components are produced by *C. sinensis* or its colonizing fungi remains to be investigated. During an ongoing search for new bioactive natural products from the *Cordyceps*-colonizing isolates,[4,5] the fungus *Trichocladium opacum* (X116), isolated from a sample of *C. sinensis* collected in Linzhi, Tibet, P. R. China, was grown in a solid-substrate fermentation culture. The extract in organic solvent displayed cytotoxicity against two human tumor cell lines, HeLa and MCF-7. Fractionation of the extract afforded three new metabolites, which we have named trichocladinols A–C (**1**–**3**), and the known compound massarigenin A (**4**; Figure 1).[6] Details of the isolation, structure elucidation, and biological activity of these metabolites are reported herein.

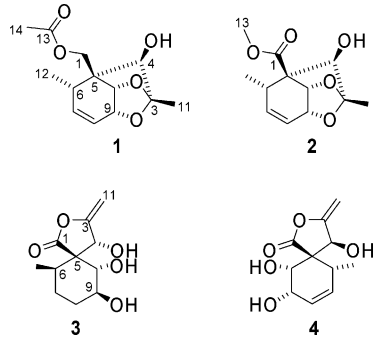

Figure 1. Metabolites **1**–**4** from *T. opacum*.

Results and Discussion

The molecular formula of trichocladinol A (**1**; atom numbering shown and used for NMR assignments not in accord with the systematic IUPAC-consistent name) was assigned as $C_{13}H_{18}O_5$ (five degrees of unsaturation) on the basis of its HRESIMS spectrum (*m*/*z* = 277.1046 [M + Na]$^+$, Δ = –0.4 mmu). Analysis of the ^{1}H, ^{13}C, and HMQC NMR spectroscopic data (Table 1) of **1** revealed one exchangeable proton (δ = 4.46 ppm), three methyl groups, one oxygenated methylene unit, four methines (three oxygenated), one disubstituted olefin, two sp^3 quaternary carbon atoms (one oxygenated), and one carboxy carbon atom. Interpretation of the ^{1}H–^{1}H COSY NMR spectroscopic data identified two isolated proton spin systems, C-6–C-10 (including C-12) and C-4–4-OH. HMBC correlations from 6-H, 7-H, 10-H, and 12-H to C-5 reveal the connection of C-5 to C-6 and C-10, completing the cyclohexene ring in **1**. Correlations from 1a-H to C-4, C-5, C-6, and C-10, and from 4-H to C-1 and C-6 indicate that both C-1 and C-4

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.200900735.

Table 1. ^1H and ^{13}C NMR spectroscopic data for **1**–**4** in [D_6]acetone and [D_6]DMSO.

Pos.	**1**		**2**		**3**	
	δ_H [ppm], (mult. J [Hz])[a]	δ_C [ppm][b]	δ_H [ppm], (mult. J [Hz])[a]	δ_C [ppm][b]	δ_H [ppm], (mult. J [Hz])[c]	δ_C [ppm][d]
1a	4.22 (d, 12)	62.4, CH_2		176.0, C_{quat}		174.0, C_{quat}
1b	4.32 (d, 12)					
3		110.6, C_{quat}		110.8, C_{quat}		155.6, C_{quat}
4	3.78 (d, 6.0)	78.4, CH	3.80 (d, 6.5)	80.1, CH	5.12 (d, 7.0)	68.1, CH
5		52.3, C_{quat}		60.9, C_{quat}		56.0, C_{quat}
6	2.56 (ddq, 3.0, 2.0, 7.5)	31.0, CH	2.80 (ddq, 3.0, 2.0, 7.5)	35.0, CH	2.10 (ddq, 6.5, 5.0, 7.0)	28.7, CH
7a	5.67 (dd, 10, 2.0)	136.6, CH	5.67 (dd, 10, 2.0)	134.9, CH	1.20 (ddd, 13, 6.5, 4.0)	28.0, CH_2
7b					2.25 (tt, 13, 5.0)	
8a	5.84 (ddd, 10, 4.5, 3.0)	124.7, CH	5.90 (ddd, 10, 4.5, 3.0)	124.6, CH	1.30 (ddd, 13, 10, 4.0)	28.1, CH_2
8b					1.60 (ddd, 13, 6.5, 5.0)	
9	3.96 (t, 4.5)	69.7, CH	3.99 (t, 4.5)	69.1, CH	3.50 (td, 10, 5.0)	70.3, CH
10	4.31 (d, 4.5)	76.4, CH	4.69 (d, 4.5)	78.4, CH	3.70 (dd, 10, 4.0)	71.4, CH
11a	1.41 (s)	14.7, CH_3	1.41 (s)	14.6, CH_3	4.53 (d, 2.5)	88.0, CH_2
11b					4.72 (d, 2.5)	
12	1.12 (d, 7.5)	16.2, CH_3	1.20 (d, 7.5)	17.6, CH_3	0.91 (d, 7.0)	16.7, CH_3
13		171.1, C_{quat}	3.76 (s)	52.8, CH_3		
14	2.04 (s)	20.8, CH_3				
OH-4	4.46 (d, 6.0)		5.19 (d, 6.5)		5.99 (d, 7.0)	
OH-9					4.63 (d, 5.0)	
OH-10					5.33 (d, 4.0)	

[a] Recorded at 500 MHz in [D_6]acetone. [b] Recorded at 100 MHz in [D_6]acetone. [c] Recorded at 500 MHz in [D_6]DMSO. [d] Recorded at 100 MHz in [D_6]DMSO.

are attached to C-5. Those from 4-OH to C-3 and from 11-H to C-3 and C-4 establish the connections of the doubly oxygenated sp^3 quaternary carbon C-3 (δ = 110.6 ppm) to C-4 and C-11. Key correlations from the oxymethine protons 9-H and 10-H to C-3 establish the tetrahydrofuran and 1,3-dioxolane fragments in **1**. In addition, NMR resonances corresponding to an acetyl group (δ = 2.04/20.8, 171.1 ppm) were observed, which indicates that the C-1 oxygen of **1** was acylated. This observation was confirmed by an HMBC cross-peak from 1-H to the carboxy carbon C-13. On the basis of these data, the gross structure of **1** was established, as shown in Figure 1.

The relative configuration of **1** was assigned by analysis of the ^1H–^1H coupling constants and NOESY data. NOESY correlation of 6-H with 10-H indicates that both protons are in pseudoaxial orientations in the cyclohexene ring. The small coupling constant of 4.5 Hz observed between 9-H and 10-H suggests a *cis* relationship, with 9-H adopting the same orientation as 6-H. NOESY correlation of 12-H with 4-H indicates that 4-H is opposite 6-H with respect to the ring system, whereas the NOESY correlation of 4-OH with 11-H reveals a *trans* relationship between 4-H and 11-H. Finally, the structure of **1** was confirmed by single-crystal X-ray diffraction analysis (Figure 2).

The modified Mosher method was applied to assign the absolute configuration of **1**.[7,8] Treatment of **1** with (*S*)- and (*R*)-MTPACl afforded the (*R*)- and (*S*)-MTPA esters **1a** and **1b**, respectively. The differences in the chemical shift values ($\Delta\delta = \delta_S - \delta_R$) for the diastereomeric esters **1b** and **1a** were calculated to assign the 4*R* absolute configuration. Therefore, the 3*R*,4*R*,5*R*,6*S*,9*R*,10*S* absolute configuration was proposed for **1** on the basis of the $\Delta\delta$ results summarized in Figure 3.

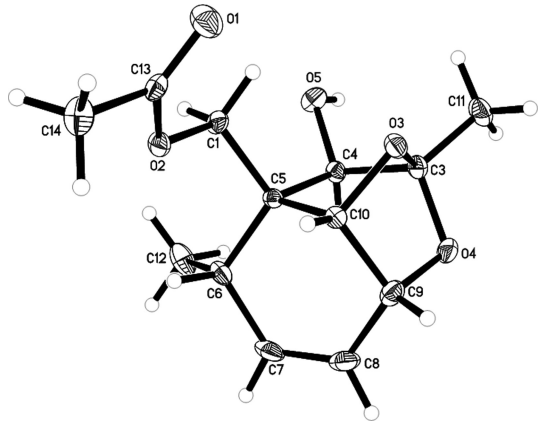
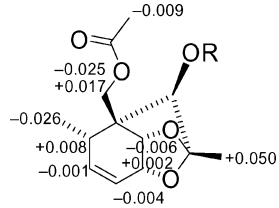


Figure 2. Thermal ellipsoid representation of **1**.



1a R = (*R*)-MTPA
1b R = (*S*)-MTPA

Figure 3. $\Delta\delta$ values ($\Delta\delta = \delta_S - \delta_R$; in ppm) obtained for (*R*)- and (*S*)-MTPA esters **1a** and **1b**.

Trichocladinol B (**2**) was assigned the molecular formula $C_{12}H_{16}O_5$ by HRESIMS analysis (*m*/*z* = 263.0898 [M + Na]$^+$, Δ = –0.8 mmu). Analysis of the ^1H and ^{13}C NMR spectroscopic data (Table 1) of **2** revealed nearly identical

structural features to those found in **1**, except that the oxygenated methylene carbon atom (C-1) in **1** is replaced by a carboxy carbon atom (δ = 176.0 ppm), and the acetyl unit is replaced by an *O*-methyl group (δ = 3.76/52.7 ppm) in the spectra of **2**. These observations were supported by HMBC correlations from 10-H and 13-H to C-1. The relative configuration of **2** was deduced to be the same as that of **1** by comparison of its ^{1}H–^{1}H coupling constants and NOESY data with those of **1**. The absolute configuration of **2** was presumed to be analogous to that of **1**.

Trichocladinol C (**3**) gives a pseudomolecular ion [M + Na]$^+$ peak at *m*/*z* = 251.0892 (Δ = –0.2 mmu) in the HRESIMS analysis, which corresponds to an elemental composition of $C_{11}H_{16}O_5$ (four degrees of unsaturation). The ^{1}H and ^{13}C NMR spectra of **3** show signals for three exchangeable protons, one methyl group, two methylene units, four methines (three oxygenated), one terminal olefin, one sp^3 quaternary carbon atom, and one carboxy carbon atom. The proton spin system corresponding to the C-6–C-10 (including C-12) subunit of **3** was established on the basis of relevant ^{1}H–^{1}H COSY correlations. HMBC cross-peaks from 6-H, 10-H, 10-OH, and 12-H to C-5 completed the cyclohexane ring in **3**. The exocyclic sp^2 methylene protons (δ = 4.53 and 4.72 ppm) are correlated to C-3 and C-4 in the HMBC spectrum of **3**, which indicates that C-4 is allylic to the C-3/C-11 terminal olefin. Correlations from 6-H and 10-H to C-4 shows the connection of C-4 to C-5, which is further supported by HMBC cross-peaks from the exchangeable proton at 4-OH (δ = 5.99 ppm) to C-3, C-4, and C-5. An HMBC correlation from 10-H to C-1 indicates that C-1 is directly attached to C-5. The remaining two exchangeable protons (δ = 4.63 and 5.33 ppm, respectively) were assigned as C-9-OH and C-10-OH on the basis of HMBC correlations from 9-OH to C-8, C-9, and C-10, and from 10-OH to C-5, C-9, and C-10. On the basis of the ^{13}C NMR chemical shifts of the C-3/C-11 exocyclic olefin (δ = 88.0 and 155.6 ppm, respectively) and the unsaturation requirement for **3**, C-3 and the carboxy carbon C-1 must be attached to the only remaining oxygen atom to form an ester linkage, thereby completing the highly functionalized 3-methylidene-2-oxaspiro[4.5]decan-1-one structure of **3**.

The relative configuration of **3** was determined by analysis of its ^{1}H–^{1}H coupling constants and NOESY data (Figure 4). The large *trans* diaxial-type coupling constant of 10 Hz observed between 9-H and 10-H indicates that they are pseudoaxially oriented, whereas the NOESY correlation of 10-H with 12-H indicates that 6-H is pseudoequatorially oriented. The NOESY correlation of 4-H with 9-H reveals their proximity in space, which suggests that the two rings of the spirocycle are nearly perpendicular to each other. Therefore, the relative configuration of trichocladinol C shown in Figure 4 was proposed.

The absolute configuration of **3** was initially assigned by application of the modified Mosher method (Figure 5). Treatment of **3** with (*S*)- and (*R*)-MTPACl afforded the (*R*)-MTPA ester **3a** and the (*S*)-MTPA esters **3b** and **3c**, respectively. However, selectivity for the acylation of one of the C-4, C-9, and C-10 hydroxy groups was not achieved, and

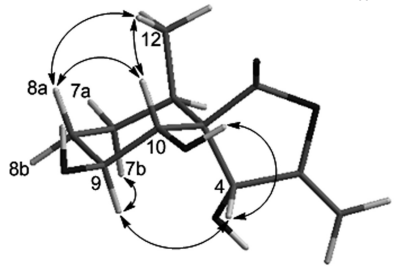


Figure 4. Key NOESY correlations for **3**.

the three esters **3a**, **3b**, and **3c** were obtained by reversed-phase HPLC purification. The calculations of all of the relevant signals of **3b** and **3a** showed anomalous values for 6-H, 7-H, and 8-H (Figure 5), possibly due to interference between the two MTPA units. Ultimately, the absolute configuration **3** was established as 4*R*,5*R*,6*R*,9*S*,10*S* by single-crystal X-ray crystallographic analysis of its (*S*)-MTPA ester **3c** (Figure 6).

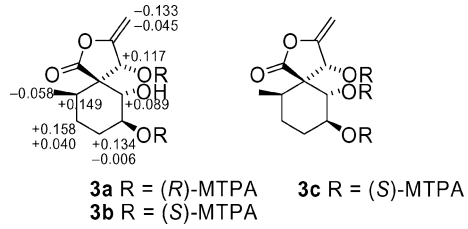


Figure 5. Structures of **3a**–**c** and $\Delta\delta$ values ($\Delta\delta = \delta_S - \delta_R$; ppm) obtained for (*R*)- and (*S*)-MTPA esters **3a** and **3b**.

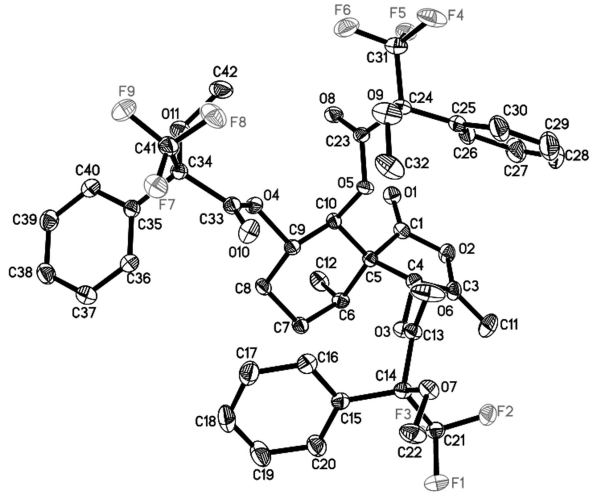


Figure 6. Thermal ellipsoid representation of **3c**.

The known compound massarigenin A (**4**) was also isolated from the crude extract, and its structure was identified by comparison of its NMR and MS data with those reported previously.[6] Massarigenin A is a metabolite pos-

sessing the 3-methylidene-2-oxaspiro[4.5]decan-1-one skeleton and was initially isolated from the aquatic fungus *Massarina tunicata* as an antimicrobial agent.

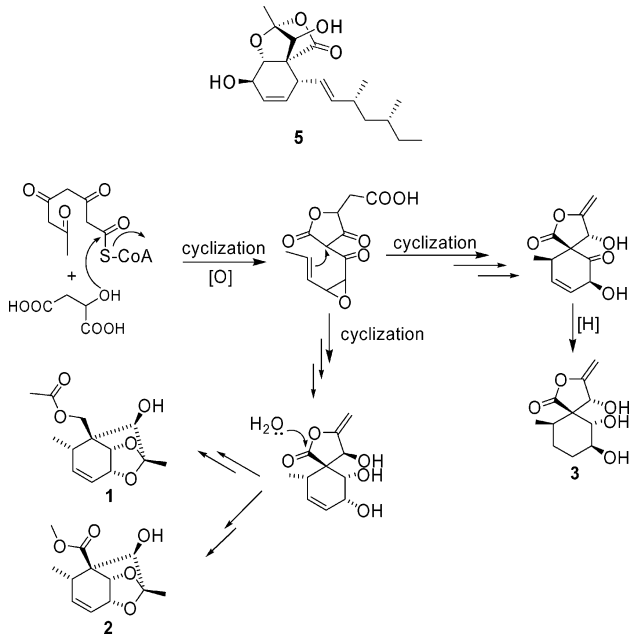
Trichocladinols A–C (**1**–**3**) were evaluated for their cytotoxicity against the human tumor cell lines HeLa and MCF-7 (Table 2). Trichocladinol B (**2**) was the most active metabolite against the two cell lines, showing IC_{50} values of 13.5 and 8.3 μM, respectively.

Table 2. Cytotoxicity of compounds **1**–**3**.

Compd.	IC_{50} [μM]	
	HeLa	MCF-7
1	118.1	59.1
2	13.5	8.3
3	43.9	65.8

Conclusions

Trichocladinols A (**1**) and B (**2**) are unique metabolites featuring an unprecedented 2,9-dioxatricyclo[5.2.1.$0^{3,8}$]dec-4-ene skeleton, the presence of the 1,3-dioxolane moiety also being relatively rare in natural products; to the best of our knowledge, only a few natural products incorporating the 1,3-dioxolane unit have been reported, for example, 6α-hydroxy-1α,4α,4,5-diepoxyxanth-10(14)-ene from the leaves of *Pluchea dioscoridis*,[9] isagarin from the roots of *Pentas longiflora*,[10] and *exo*-7-ethyl-5-methyl-6,8-dioxabicyclo-[3.2.1]oct-3-ene from the urine of adult male mice as a chemical signal of the male state.[11] Although the known compound spiroleptosphol B (**5**) possesses the same hexahydrobenzofuran substructure and substitution pattern as **1** and **2**, it possesses a different 7,9-dioxatricyclo[6.2.1.$0^{1,6}$]-undec-3-en-10-one skeleton.[12] These metabolites may share the same biosynthetic processors on the basis that the 2,9-dioxatricyclo[5.2.1.$0^{3,8}$]dec-4-ene skeleton in **1** and **2** could be formed from the 7,9-dioxatricyclo[6.2.1.$0^{1,6}$]undec-3-en-10-one skeleton or vice versa through rearrangement reactions. Trichocladinol C (**3**) is closely related to massarigenin A (**4**),[6] both belonging to the γ-methylidene-spirobutanolide class of metabolites, which have mainly been isolated from the basidiomycetous and ascomycetous fungi.[12–21] Biogenetically, by comparison of the biogenesis of arthropsolide A,[20] the γ-methylidene-spirobutanolides could be derived from the condensation of a tetraketide precursor and a malic acid unit followed by a series of reactions including reduction, elimination, and epoxidation. The carbon skeleton could also be derived from a polyketide chain through some unusual rearrangement reactions.[21] The possible biosynthetic pathways of trichocladinols A–C (**1**–**3**) are proposed in Scheme 1. Compounds **1**–**4** are the first natural products to be reported from the *Cordyceps*-colonizing ascomycete *Trichocladium opacum*, and the discovery of these metabolites implies that *Cordyceps*-colonizing fungi could be a useful source of diverse bioactive natural products.



Scheme 1. Proposed biogenesis of **1**–**3**.

Experimental Section

General: Optical rotations were measured with a Perkin–Elmer 241 polarimeter and UV data were recorded with a Shimadzu Biospec-1601 spectrophotometer. The CD spectra were recorded with a JASCO J-815 spectropolarimeter using MeOH as the solvent. IR data were recorded using a Nicolet Magna-IR 750 spectrometer. ^1H and ^{13}C NMR spectroscopic data were acquired with Varian Mercury-400 and Inova-500 spectrometers using the solvent signals as references ([D_6]acetone: δ = 2.05/29.8, 206.0 ppm; [D_6]DMSO: δ = 2.50/39.5 ppm). The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS and HRESIMS data were recorded with a Mariner ESI-TOF mass spectrometer.

Fungal Material: The culture of *Trichocladium opacum* was isolated by Dr. Mu Wang from a sample of *C. sinensis* (Berk.) Sacc. collected in Linzhi, Tibet, on March 1, 2004. The isolate was identified based on sequence (Genbank accession number GQ179993) analysis of the ITS region of the rDNA and assigned the accession number X116 in the culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The isolate was subcultured on slants of potato dextrose agar (PDA) at 25 °C for 5 d. Fermentation was carried out in six 500-mL Erlenmeyer flasks, each containing 80 g of rice. Distilled H_2O (120 mL) was added to each flask and the contents were soaked overnight before autoclaving at 15 psi for 30 min. The flasks were cooled to room temperature and inoculated with 3.0 mL of a hyphal cell suspension and incubated at 25 °C for 40 d.

Extraction and Isolation: The fermented rice substrate was extracted repeatedly with EtOAc (3 × 1.0 L) and the organic solvent was evaporated to dryness under a vacuum to afford a crude extract (6.0 g) that was fractionated by silica gel vacuum column chromatography (CC; 5 × 8 cm) using petroleum ether/EtOAc gradient elution. The fraction eluted with 25% EtOAc (200 mg) was further separated by Sephadex LH-20 CC eluting with MeOH. One subfraction (50 mg) was further separated by semipreparative RPHPLC (Agilent Zorbax SB-C_{18} column, 5 μm, 9.4 × 250 mm, 2 mL min^{-1}) to afford trichocladinol A (**1**; 15.0 mg, t_R = 14.25 min;

45% MeOH in H_2O for 5 min followed by 45–60% for 25 min). The fractions (1.0 g) eluted with 30–45% EtOAc were combined and fractionated by Sephadex LH-20 CC using CH_2Cl_2/MeOH (1:1) as eluent, and one of the subfractions (800 mg) was separated again by silica gel CC (2 × 13 cm) eluting with CH_2Cl_2/MeOH gradients. One resulting subfraction (50 mg) eluted with 1% MeOH was purified by RPHPLC to afford trichocladinol C (**3**; 10.0 mg, t_R = 18.70 min; 35% MeOH in H_2O for 5 min followed by 35–50% for 25 min) and another subfraction (20 mg) also eluted with 1% MeOH was purified by RPHPLC using a different gradient (40% MeOH in H_2O for 5 min followed by 40–60% for 25 min) to afford trichocladinol B (**2**; 4.0 mg, t_R = 14.30 min) and massarigenin A (**4**; 8.0 mg, t_R = 16.00 min).

Trichocladinol A [(10-Hydroxy-1,6-dimethyl-2,9-dioxatricyclo-[5.2.1.0^{3,8}]dec-4-en-7-yl)methyl Acetate] (1): Colorless oil. $[\alpha]_D^{22}$ = +78 (*c* = 0.03, CH_3OH). UV (CH_3OH): λ_{max} [log(ε/M^{-1}cm^{-1})] = 200 [3.62] nm. IR (neat): $\tilde{\nu}_{max}$ = 3465 (br), 2974, 1739, 1453, 1382, 1242 cm^{-1}. For ^{1}H and ^{13}C NMR spectroscopic data, see Table 1. HMBC data (400 MHz, [D$_6$]acetone, 25 °C): 1a-H → C-4, C-5, C-6, C-10, C-13; 1b-H → C-13; 4-H → C-1, C-6; 6-H → C-4, C-5, C-7, C-8, C-12; 7-H → C-5, C-6, C-8, C-9, C-12; 8-H → C-6, C-7, C-9, C-10; 9-H → C-3, C-5, C-7, C-10; 10-H → C-3, C-4, C-5, C-6, C-9; 11-H → C-3, C-4; 12-H → C-5, C-6, C-7; 14-H → C-13; 4-OH → C-3, C-4, C-5. NOESY correlations (400 MHz, [D$_6$]acetone, 25 °C): 4-H ↔ 12-H; 6-H ↔ 10-H; 4-OH ↔ 11-H. HRMS (ESI): calcd. for $C_{13}H_{18}O_5Na$ [M + Na]$^+$ 277.1046; found 277.1050.

X-ray Crystallographic Analysis of 1: Upon crystallization from acetone/H_2O (10:1) using the vapor diffusion method, colorless crystals were obtained for **1** and a crystal (0.58 × 0.51 × 0.45 mm) was separated from the sample and mounted on a glass fiber, and data were collected using a Rigaku R-AXIS RAPID IP diffractometer with graphite-monochromated Mo-K_α radiation(λ = 0.71073 Å) at 173(2) K. Crystal data: $C_{13}H_{18}O_5$, *M* = 254.27, space group monoclinic, *P*21, unit cell dimensions: *a* = 7.3075(15), *b* = 9.2713(19), *c* = 10.017(2) Å, *V* = 638.1(2) Å^3, *Z* = 2, $D_{calcd.}$ = 1.323 mg m^{-3}, μ = 0.101 mm^{-1}, *F*(000) = 272. The structure was solved by direct methods using SHELXL-97[22] and refined by using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined with anisotropic displacement parameters and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. Absorption corrections were performed by using the Siemens Area Detector Absorption Program (SADABS).[23] The 5238 measurements yielded 1555 independent reflections after equivalent data had been averaged and Lorentz and polarization corrections applied. The final refinement gave R_1 = 0.0476 and wR_2 = 0.1132 [$I > 2\sigma(I)$].[24]

Preparation of (*R*)- and (*S*)-MTPA Esters 1a and 1b: A sample of **1** (2.0 mg, 0.008 mmol) was dissolved in CH_2Cl_2 (3.0 mL) in a 10-mL round-bottomed flask. DMAP (10.0 mg) and (*S*)-MTPACl (10.0 μL, 0.052 mmol) were quickly added and the flask was sealed and the contents stirred at room temperature for 24 h. The mixture was evaporated to dryness and purified by semipreparative RPHPLC (Agilent Zorbax SB-C$_{18}$ column, 5 μm, 9.4 × 250 mm; 80% CH_3OH in H_2O for 5 min followed by 80–100% for 25 min, 2 mL min^{-1}) to afford **1a** (1.2 mg, t_R = 16.30 min) as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 5.94 (ddd, *J* = 10, 4.0, 3.0 Hz, 1 H, 8-H), 5.71 (dd, *J* = 10, 2.0 Hz, 1 H, 7-H), 5.19 (s,1 H, 4-H), 4.45 (d, *J* = 4.0 Hz, 1 H, 10-H), 4.13 (t, *J* = 4.0 Hz, 1 H, 9-H), 4.07 (d, *J* = 12 Hz, 1 H, 1b-H), 3.99 (d, *J* = 12 Hz, 1 H, 1a-H), 2.49 (ddq, *J* = 7.5, 3.0, 2.0 Hz, 1 H, 6-H), 2.05 (s, 3 H, 14-H), 1.32 (s, 3 H, 11-H), 1.00 (d, *J* = 7.5 Hz, 3 H, 12-H) ppm.

In a similar fashion, a sample of **1** (2.0 mg, 0.008 mmol), CH_2Cl_2 (3.0 mL), DMAP (10.0 mg), and (*R*)-MTPACl (10.0 μL, 0.052 mmol) were allowed to react in a 10-mL round-bottomed flask at room temperature for 18 h and the reaction mixture was processed as described above for **1a** to afford **1b** (1.3 mg) as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 5.93 (ddd, *J* = 10, 4.0, 3.0 Hz, 1 H, 8-H), 5.71 (dd, *J* = 10, 2.0 Hz, 1 H, 7-H), 5.18 (s, 1 H, 4-H), 4.44 (d, *J* = 4.0 Hz, 1 H, 10-H), 4.13 (t, *J* = 4.0 Hz, 1 H, 9-H), 4.08 (d, *J* = 12 Hz, 1 H, 1b-H), 3.96 (d, *J* = 12 Hz, 1 H, 1a-H), 2.50 (ddq, *J* = 7.5, 3.0, 2.0 Hz, 1 H, 6-H), 2.04 (s, 3 H, 14-H), 1.37 (s, 3 H, 11-H), 0.98 (d, *J* = 7.5 Hz, 3 H, 12-H) ppm.

Trichocladinol B (2): Colorless oil. $[\alpha]_D^{22}$ = +141 (*c* = 0.06, CH_3OH). UV (CH_3OH): λ_{max} [log(ε/M^{-1}cm^{-1})] = 203 [4.24] nm. IR (neat): $\tilde{\nu}_{max}$ = 3426 (br), 2942, 1708, 1439, 1393 cm^{-1}. For ^{1}H and ^{13}C NMR spectroscopic data, see Table 1. HMBC data (400 MHz, [D$_6$]acetone, 25 °C): 4-H → C-6; 6-H → C-4, C-7, C-8, C-12; 7-H → C-5, C-6, C-8, C-9, C-12; 9-H → C-5, C-7, C-10; 10-H → C-1, C-3, C-4, C-9; 11-H → C-3, C-4; 12-H → C-5, C-6, C-7; 13-H → C-1. NOESY correlations (400 MHz, [D$_6$]acetone, 25 °C): 4-H ↔ 12-H; 9-H ↔ 11-H. HRMS (ESI): calcd. for $C_{12}H_{16}O_5Na$ [M + Na]$^+$ 263.0890; found 263.0898.

Trichocladinol C (3): Colorless oil. $[\alpha]_D^{22}$ = +60 (*c* = 0.07, CH_3OH). UV (CH_3OH) λ_{max} [log(ε/M^{-1}cm^{-1})] = 204 [3.95] nm. IR (neat): $\tilde{\nu}_{max}$ = 3489, 3320 (br), 2970, 1789, 1692, 1463, 1392 cm^{-1}. For ^{1}H and ^{13}C NMR spectroscopic data, see Table 1. HMBC data (400 MHz, [D$_6$]DMSO, 25 °C): 4-H → C-3, C-5, C-6, C-10, C-11; 6-H → C-4, C-5, C-8, C-10, C-12; 7a-H → C-8; 7b-H → C-5, C-8, C-12; 8a-H → C-7, C-9; 8b-H → C-7; 9-H → C-10; 10-H → C-1, C-4, C-5, C-9; 11-H → C-3, C-4; 12-H → C-5, C-6, C-7; 4-OH → C-3, C-4, C-5; 9-OH → C-8, C-9, C-10; 10-OH → C-5, C-10. NOESY correlations (400 MHz, [D$_6$]DMSO, 25 °C): 4-H ↔ 10-OH; 8a-H ↔ 12-H; 9-H ↔ 4-H, 7b-H; 10-H ↔ 8a-H, 12-H. HRMS (ESI): calcd. for $C_{11}H_{16}O_5Na$ [M + Na]$^+$ 251.0890; found 251.0892.

Preparation of the (*R*)-MTPA Ester 3a and the (*S*)-MTPA Esters 3b and 3c: A sample of **3** (2.0 mg, 0.009 mmol) was dissolved in CH_2Cl_2 (4.0 mL) in a 10-mL round-bottomed flask. DMAP (10.0 mg) and (*S*)-MTPACl (10.0 μL, 0.052 mmol) were quickly added and the flask was sealed and the contents stirred at room temperature for 24 h. The mixture was evaporated to dryness and purified by semipreparative RPHPLC (Agilent Zorbax SB-C$_{18}$ column, 5 μm, 9.4 × 250 mm, 80% CH_3OH in H_2O for 5 min followed by 80–100% for 25 min, 2 mL min^{-1}) to afford **3a** (1.2 mg, t_R = 22.50 min) as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 6.47 (s,1 H, 4-H), 4.97 (d, *J* = 1.5 Hz, 1 H, 11b-H), 4.71 (td, *J* = 10, 4.0 Hz, 1 H, 9-H), 4.68 (d, *J* = 1.5 Hz, 1 H, 11a-H), 4.14 (d, *J* = 10 Hz, 1 H, 10-H), 2.25 (tt, *J* = 13, 5.0 Hz, 1 H, 7b-H), 2.07 (ddd, *J* = 7.0, 6.5, 5.0 Hz, 1 H, 6-H), 1.68 (ddd, *J* = 13, 6.5, 5.0 Hz, 1 H, 8b-H), 1.30 (ddd, *J* = 13, 10, 4.0 Hz, 1 H, 8a-H), 1.16 (ddd, *J* = 13, 6.5, 4.0 Hz, 1 H, 7a-H), 1.08 (d, *J* = 7.0 Hz, 3 H, 12-H) ppm.

In a similar fashion, a sample of **3** (2.0 mg, 0.009 mmol), CH_2Cl_2 (4.0 mL), DMAP (10.0 mg), and (*R*)-MTPACl (10.0 μL, 0.052 mmol) were allowed to react in a 10-mL round-bottomed flask at room temperature for 18 h and the reaction mixture was processed as described above for **3a** to afford **3b** (1.3 mg) and **3c** (0.9 mg) as white powders. **3b**: ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 6.58 (s, 1 H, 4-H), 4.92 (d, *J* = 1.5 Hz, 1 H, 11b-H), 4.70 (td, *J* = 10, 4.0 Hz, 1 H, 9-H), 4.55 (d, *J* = 1.5 Hz, 1 H, 11a-H), 4.22 (d, *J* = 10 Hz, 1 H, 10-H), 2.29 (tt, *J* = 13, 5.0 Hz, 1 H, 7b-H), 2.20 (ddd, *J* = 7.0, 6.5, 5.0 Hz, 1 H, 6-H), 1.67 (ddd, *J* = 13, 6.5, 5.0 Hz, 1 H, 8b-H), 1.44 (ddd, *J* = 13, 10, 4.0 Hz, 1 H, 8a-H), 1.32

(ddd, J = 13, 6.5, 4.0 Hz, 1 H, 7a-H), 1.02 (d, J = 7.0 Hz, 3 H, 12-H) ppm. **3c**: ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 6.30 (s, 1 H, 4-H), 5.82 (d, J = 10 Hz, 1 H, 10-H), 4.82 (td, J = 10, 4.0 Hz, 1 H, 9-H), 4.71 (d, J = 1.5 Hz, 1 H, 11b-H), 4.38 (d, J = 1.5 Hz, 1 H, 11a-H), 2.20 (tt, J = 13, 5.0 Hz, 1 H, 7b-H), 1.60 (ddd, J = 7.0, 6.5, 5.0 Hz, 1 H, 6-H), 1.42 (ddd, J = 13, 6.5, 5.0 Hz, 1 H, 8b-H), 1.20 (ddd, J = 13, 10, 4.0 Hz, 1 H, 8a-H), 1.08 (ddd, J = 13, 6.5, 4.0 Hz, 1 H, 7a-H), 0.99 (d, J = 7.0 Hz, 3 H, 12-H) ppm.

X-ray Crystallographic Analysis of 3c: Upon crystallization from MeOH using the vapor diffusion method, colorless crystals were obtained for **3c** and a crystal (0.26 × 0.17 × 0.03 mm) was separated from the sample and mounted on a glass fiber. The data were collected by using a Rigaku MicroMax-007HF Saturn 724+ CCD diffractometer with Mo-K_α radiation (λ = 0.71073 Å) at 173(2) K. Crystal data: $C_{41}H_{37}F_9O_{11}$, M = 876.71, space group triclinic, $P\bar{1}$, unit cell dimensions a = 8.3882(17), b = 11.056(2), c = 21.830(4) Å, V = 2007.0(7) Å^3, Z = 2, $D_{calcd.}$ = 1.451 mg m^{-3}, μ = 0.131 mm^{-1}, F(000) = 904. The structure was solved by direct methods using SHELXL-97[22] and refined by using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined with anisotropic displacement parameters and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. Absorption corrections were performed by using the Siemens Area Detector Absorption Program (SADABS).[23] The 33313 measurements yielded 9164 independent reflections after equivalent data had been averaged and Lorentz and polarization corrections applied. The final refinement gave R_1 = 0.0547 and wR_2 = 0.1203 [$I > 2\sigma(I)$].[24]

Massarigenin A (4): The ^{1}H NMR, ^{13}C NMR, and ESIMS data were fully consistent with literature values.[6]

MTT Assay:[4] In 96-well plates, 10^4 cells were introduced into each well. After cell attachment overnight, the medium was removed and each well was treated with 50 μL of medium containing 0.2% DMSO or the appropriate concentration of test compounds (10 mg mL^{-1} as the stock solution of the compound in DMSO and serial dilutions). Cells were first treated at 37 °C for 4 h in a humidified incubator at 5% CO_2 and then grown for another 48 h after the medium had been changed to fresh Dulbecco's Modified Eagle Medium (DMEM). MTT (Sigma) was dissolved in a serum-free medium or PBS at 0.5 mg mL^{-1} and sonicated briefly. In the dark, 50 μL of MTT/medium was added to each well after the medium had been removed from the wells and incubated at 37 °C for 3 h. Upon removal of the MTT/medium, 100 μL of DMSO was added to each well and agitated at 60 rpm for 5 min to dissolve the precipitate. The assay plate was read at 540 nm by using a microplate reader.

Supporting Information (see also the footnote on the first page of this article): ^{1}H and ^{13}C NMR spectra of trichocladinols A–C (**1**–**3**).

Acknowledgments

We gratefully acknowledge financial support from the Ministry of Science and Technology of China (2009CB522302) and the National Natural Science Foundation of China (30870057 and U0633008).

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[24] CCDC-734059 (for **1**) and -734060 (for **3c**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Received: July 3, 2009
Published Online: September 24, 2009

