Cytosporinols A−C, new caryophyllene sesquiterpenoids from *Cytospora* sp.

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Abstract: Three new caryophyllene sesquiterpenoids, cytosporinols A–C (**1**–**3**), have been isolated from solid cultures of *Cytospora* sp. The structures of **1**–**3** were elucidated primarily by NMR spectroscopy, and **3** was further confirmed by X-ray crystallography. The absolute configurations of the C-11 secondary alcohol in **1** and the 6,8-diol moiety in **3** were deduced using the modified Mosher and Snatzke's method, respectively. Compounds **2** and **3** showed moderate cytotoxicity against HeLa cells.

Keywords: caryophyllene sesquiterpenoid, cytosporinol, *Cytospora* sp.

Introduction

Caryophyllene sesquiterpenoids have been isolated frequently from fungi as the bioactive principles. Examples include the pestalotiopsins, immunosuppressive and cytotoxic agents produced by the endophyte *Pestalotiopsis* sp. isolated from *Taxus brevifolia*;[1,2] pestalotiopsolide A, teadolidol, and 6-epiteadolidol, highly oxidized caryophyllenes from a *Pestalotiopsis* sp. endophytic to the bark of *Pinus taeda*;[3] the 6-hydroxpunctaporonins, antibacterial metabolites from a fungicolous *Pestalotiopsis disseminata*;[4] fuscoatrol A, a cytotoxic agent from a marine-derived *Humicola fuscoatra* KMM 4629;[5] the punctaporonins (also named as punctatins), antifungal agents from a coprophilous *Poronia punctata*;[6–11] walleminol and walleminone, two *cis*-fused iso-caryophyllenes from the toxigenic fungus *Wallemia sebi*;[12] and Sch 725432, 601253, 601254, and 725434, antifungal agents from *Chrysosporium pilosum*.[13]

During an ongoing search for new bioactive natural products from fungi of unique habitats, we initiated chemical studies of the fungi either inhabiting the fruiting body and larvae of *Cordyceps sinensis*[14] or its surface soil.[15] As an extension, we also studied those species isolated from the soil samples that were collected on the Qinghai-Tibetan plateau at altitudes above 3,200 m, the environment in which *Cordyceps sinensis* was typically found. Our initial investigation of such an ascomycetous fungus *Cytospora* sp. led to the isolation of three antimicrobial caryophyllene-derived meroterpenoids.[16] Since the HPLC chromatogram of the crude extract revealed the presence of some minor components that could be the
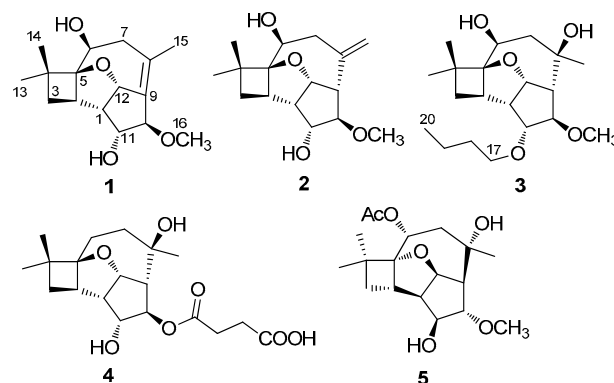
cytotoxic principles remained to be identified, the fungus was refermented in a larger scale using the same solid fermentation culture medium in which the meroterpenoids were first isolated.[16] Fractionation of an EtOAc extract afforded three new caryophyllene sesquiterpenoids which we named cytosporinols A–C (**1**–**3**). Details of the isolation, structure elucidation, and cytotoxicity of these compounds are described herein.

Results and Discussion

Cytosporinol A (**1**) was assigned the molecular formula $C_{16}H_{24}O_4$ (five degrees of unsaturation) by HRESIMS. Analysis of its NMR data (Table 1) revealed two exchangeable protons (δ_H 3.37 and 3.93, respectively), four methyl groups (one *O*-methyl), two methylenes, six methines including four oxymethines, two sp^3 quaternary carbons (one oxygenated), and one tetrasubstituted olefin. These data accounted for all the ^1H and ^{13}C NMR resonances, suggesting that **1** was a tetracyclic compound. The ^1H–^1H COSY NMR data showed

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Table 1. NMR data (400 MHz, acetone-d_6) for 1

pos.	δ_H (*J* in Hz)	δ_C, type	HMBC[a]
1	2.44, ddd (7.4, 6.5, 2.9)	56.1, CH	3, 9, 10
2	2.98, td (8.1, 2.9)	39.9, CH	
3a	1.83, dd (11.1, 8.1)	43.5, CH$_2$	1, 2, 4, 5, 14
3b	1.36, dd (11.1, 8.1)		1, 2, 14
4		38.7, qC	
5		95.5, qC	
6	4.04, ddd (12.0, 7.4, 4.8)	77.5, CH	2
7a	3.22, t (12.0)	39.7, CH$_2$	5, 6, 8, 9, 15
7b	1.85, dd (12.0, 7.4)		5, 6, 8, 9, 15
8		130.2, qC	
9		134.5, qC	
10	3.82, s	86.4, CH	1, 8, 11, 12, 16
11	4.02, dd (7.4, 4.3)	73.8, CH	2, 9, 10
12	5.10, d (6.5)	89.6, CH	2, 5, 8
13	1.36, s	27.9, CH$_3$	3, 4, 5, 14
14	1.02, s	24.1, CH$_3$	3, 4, 5, 13
15	1.74, s	22.1, CH$_3$	7, 8, 9
16	3.12, s	55.0, CH$_3$	10
OH-6	3.37, d (4.8)		5, 6
OH-11	3.93, d (4.3)		

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

two isolated spin-systems of C-10–C-1–C-3 (including OH-11 and C-12) and C-6–C-7 (including OH-6). Interpretation of the HMBC data of **1** established a cyclobutane partial structure with two methyl groups attached to C-4. HMBC cross-peaks from H-6 to C-2 and from H$_2$-7 to C-5 indicated that C-6 is attached to the cyclobutane ring at C-5. The H$_2$-7 protons were correlated to C-8, C-9, and C-15, while the Me-15 protons were correlated to C-7, C-8, and C-9 in the HMBC spectrum of **1**, linking the C-8 olefinic carbon (δ_C 130.2) to C-7, C-9 (δ_C 134.5), and C-15. HMBC correlations from H-10 to C-8 and C-12, H-11 to C-9, and from H-12 to C-8 connected C-9 to both C-10 and C-12, completing the partial structure with a cyclopentane moiety fused to a cyclooctene unit at C-1/C-9. The key HMBC correlation from H-12 to C-5 (δ_C 95.5) connected both C-5 and C-12 (δ_C 89.6) to the same oxygen atom to form a tetrahydrofuran (THF) ring fused to the cyclobutane and cyclopentane moieties at C-2/C-5 and C-1/C12, respectively. The HMBC correlation from the *O*-methyl proton signal Me-16 (δ_H 3.12) to the C-10 oxymethine carbon (δ_C 86.4) located the only methoxy group at C-10. On the basis of these data, the planar structure of **1** was established.

The relative configuration of **1** was deduced by analysis of the ^{1}H–^{1}H coupling constants and NOESY data (Figure 1). The vicinal coupling constants observed for H-1 with H-11 and H-12 were 7.4 and 6.5 Hz, respectively, suggesting their
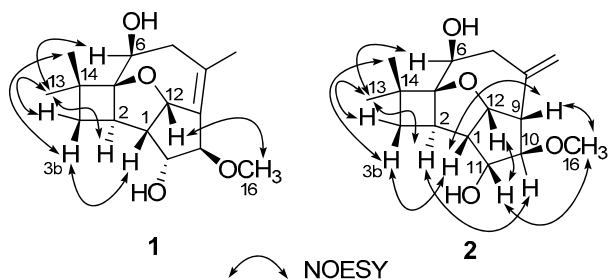

Figure 1. Key NOESY correlations for cytosporinols A (**1**) and B (**2**)

cis-relative orientation,[17] consistent with a similar THF moiety found in the known compound punctaporonin C (**4**).[9,18] NOESY correlations of Me-13 with H-2, H-3a, and H-6 indicated that these protons are all on the same face of the ring system, whereas those of H-3b with H-1 and Me-14, and of H-12 with Me-16 were used to place them on the opposite face. Collectively, these data allowed assignment of the relative configuration of **1**. The 1*S*, 2*R*, 5*S*, 6*S*, 10*R*, 11*R*, and 12*S* absolute configurations were deduced for the stereogenic centers in **1** using the modified Mosher method[19] as illustrated in Figure 2, which were also consistent with those assigned for corresponding stereogenic centers in **4**.[18]
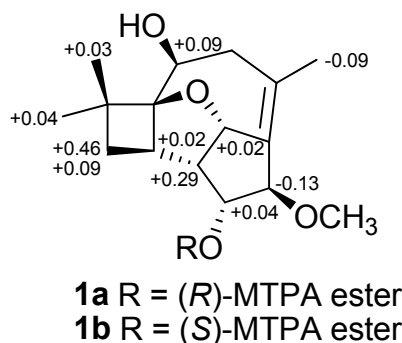

1a R = (*R*)-MTPA ester
1b R = (*S*)-MTPA ester

Figure 2. $\Delta\delta$ values (in ppm) = $\delta_S - \delta_R$ obtained for (*R*)- and (*S*)-MTPA esters **1a** and **1b**

Cytosporinol B (**2**) was assigned the same molecular formula C$_{16}$H$_{24}$O$_4$ as **1** by HRESIMS. Analysis of its NMR data (Table 2) revealed structural fragments similar to those presented in **1**. However, the C-8–C-15 unit and the C-9 olefinic carbon (δ_C 134.5) were replaced by a terminal olefin (δ_H/δ_C 4.87/117.3, 144.7) and a methine (δ_H/δ_C 2.90/58.0), respectively, which were supported by relevant ^{1}H–^{1}H COSY and HMBC correlations. Therefore, the planar structure of **2** was proposed as shown. The relative configuration of **2** was also determined by analysis of the ^{1}H–^{1}H coupling constants and NOESY data (Figure 1). The coupling patterns and NOESY correlations for the relevant protons in **2** were nearly identical to those of **1**, indicating that they possess the same relative configuration, except for the additional stereogenic center C-9, which was assigned by comparison of the coupling constant between H-9 and H-12 (9.7 Hz) with that reported for **4**.[9,18] This assignment was supported by NOESY correlations of H-9 with H-1 and Me-16. Considering the absolute configuration established for **1** by the modified Mosher method, C-9 was deduced to have the *S* configuration.

Cytosporinol C (**3**) gave a pseudomolecular ion [M + Na]$^+$ peak by HRESIMS, consistent with a molecular formula of C$_{20}$H$_{34}$O$_5$ (four degrees of unsaturation). Analysis of its NMR data (Table 2) indicated that it is closely related to **1** and **2**, except that the C-8/C-9 olefin in **1** or the C-8/C-15 terminal olefin in **2** was hydrated, and the OH-11 exchangeable proton (δ_H 3.93 in **1**; 4.28 in **2**) was replaced by a *n*-butyl group. These observations were supported by HMBC correlations from Me-15 to C-7, C-8, and C-9, and from the oxymethine proton H-11 to C-17, respectively. The relative configuration of **3** was assigned on the basis of NOESY data, and was confirmed by single crystal X-ray crystallographic analysis

Table 2. NMR data for 2 (acetone-d_6) and 3 (CDCl$_3$)

	cytosporinol B (2)			cytosporinol C (3)			
pos.	δ_H^a (*J* in Hz)	δ_C^b, type	HMBC^c	δ_H^a (*J* in Hz)	δ_C^b, type	HMBC^c	NOESY^d
1	2.74, ddd (7.0, 5.8, 3.0)	60.1, CH	3, 9, 10	2.89, td (7.5, 3.3)	56.8, CH	3, 9, 10	3b
2	2.78, ddd (9.4, 6.2, 3.0)	34.8, CH	3	2.46, td (9.8, 3.3)	33.5, CH	4, 6, 11	10
3a	1.87, dd (11.6, 9.4)	41.2, CH$_2$	1, 2, 4, 5, 13	1.95, dd (11.3, 9.8)	40.2, CH$_2$	1, 2, 4, 5, 13, 14	
3b	1.32, dd (11.6, 6.2)		1, 2, 4, 5, 13, 14	1.36, dd (11.3, 9.8)		1, 2, 4, 5, 13, 14	1, 14
4		37.5, qC			37.3, qC		
5		96.0, qC			95.4, qC		
6	3.93, d t (11.9, 3.2)	68.4, CH		4.16, br	71.4, CH	8	15
7a	2.65, dd (14.0, 3.2)	38.6, CH$_2$	8, 15	2.05, dd (16.0, 3.5)	40.3, CH$_2$	9	10
7b	2.19, dd (14.0, 3.2)		15	1.85, dd (16.0, 3.5)		5, 6, 8, 9, 15	
8		144.7, qC			76.0, qC		
9	2.90, dd (9.7, 5.0)	58.0, CH	8, 10, 12	2.25, t (7.5)	56.9, CH	1, 7, 8, 10, 12, 15	
10	3.58, t (5.0)	92.1, CH	8, 11, 12, 16	3.26, t (7.5)	87.7, CH	8, 9, 11, 16	2, 7a, 15, 17
11	4.04, ddd (5.8, 5.0, 4.3)	76.3, CH	2, 10	3.72, t (7.5)	84.4, CH	2, 10, 17	16
12	4.81, dd (9.7, 7.0)	84.8, CH	2, 5	4.98, t (7.5)	83.9, CH	2, 5, 8	
13	1.17, s	26.7, CH$_3$	3, 4, 5, 14	1.21, s	26.4, CH$_3$	3, 4, 5, 14	
14	0.97, s	24.6, CH$_3$	3, 4, 5, 13	1.08, s	24.5, CH$_3$	3, 4, 5, 13	3b
15a	4.87, d (4.9)	117.3, CH$_2$	7, 9	1.19, s	30.0, CH$_3$	7, 8, 9	6, 10
15b	4.81, d (4.9)		7, 9				
16	3.27, s	57.5, CH$_3$	10	3.35, s	57.5, CH$_3$	10	11
17				3.34, t (6.3)	69.4, CH$_2$	11, 18, 19	10
18				1.52, m	32.0, CH$_2$	17, 19, 20	
19				1.35, m	19.4, CH$_2$	17, 18, 20	
20				0.90, t (7.4)	13.8, CH$_3$	18, 19	
OH-6	2.32, d (11.9)			3.37, d (4.8)		5, 6	
OH-11	4.28, d (4.3)						

^{a}Recorded at 500 MHz; ^{b}Recorded at 100 MHz; ^{c}HMBC correlations, optimized for 8 Hz, are stated from proton(s) to the indicated carbons; ^{d}Recorded at 400 MHz.
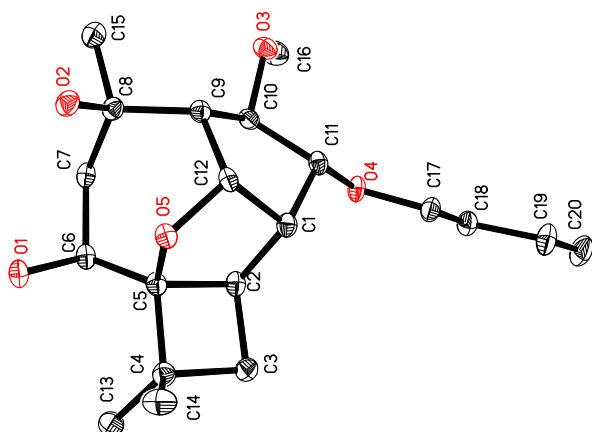
(Figure 3).

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

The absolute configuration of the *cis*-6,8-diol moiety in **3** was assigned using the in situ dimolybdenum CD method developed by Frelek.[20,21] Upon addition of dimolybdenum tetraacteate [Mo$_2$(OAc)$_4$] to a solution of **3** in DMSO, a metal complex was generated as an auxiliary chromophore. Due to the absence of other chromophores in **3**, the observed sign of the Cotton effect in the induced spectrum originates solely from the chirality of the 6,8-diol moiety expressed by the sign of the "parallel" mode for Mo-O-C-C-C-O-Mo.[22] When the molecule is viewed along the O-C bonds (Figure 4), the upper left and lower right sectors are defined as negative, whereas the upper right and lower left ones are defined as positive. The predicted sign of the Cotton effect depends upon the sign of the sector occupied by the largest part of the molecule. The negative Cotton effect observed at around 400 nm (band II) in

the induced CD spectrum of **3** (Figure 5) permitted assignment of the 6*S* and 8*R* absolute configuration on the basis of the empirical rule proposed by Snatzke.[20] Therefore, **3** was assigned 1*R*, 2*R*, 5*S*, 6*S*, 8*R*, 9*S*, 10*R*, 11*R*, and 12*S* absolute configuration.
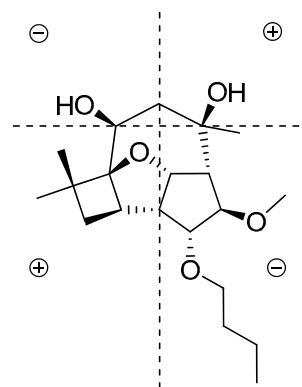

Figure 4. Projection of the sector rule for the 6,8-diol moiety in **3**

Compounds **2** and **3** showed modest cytotoxicity against HeLa (cervical epithelium) cells, showing IC$_{50}$ values of 16.5 and 21.1 μM, respectively, while the positive control cisplatin showed an IC$_{50}$ value of 7.6 μM. However, compound **1** did not show noticeable cytotoxic effects against HeLa cells (IC$_{50}$ > 100 μM).

Cytosporinols A–C (**1–3**) are closely related to the known taedolidol,[3] 6-epitaedolidol,[3] and punctaporonin C (**4**),[7–9] all possessing the rare oxatetracyclo[6.3.2.0^{1,4}.0^{5,13}]tridecane skeleton, but differ by having different substituents at C-6 (**1**), C-10 (**1–3**), and C-11 (**3**), as well as a tetrasubstituted olefin (**1**) and a terminal olefin (**2**). In addition, **1–3** are structurally similar to a synthetic byproduct **5** created in the acid-mediated
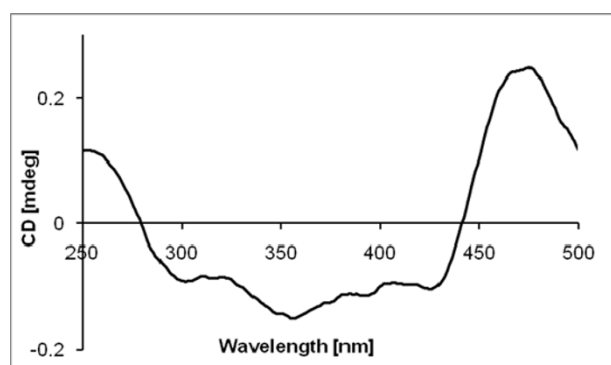
Figure 5. CD spectrum of **3** in DMSO containing Mo₂(OAc)₄

cyclization of the nonatural enantiomer pestalotiopins,[23,24] but differ in having different configurations for all stereogenic centers and substituents at C-6 and C-11, respectively. Biogenetically, **1**–**3** could be derived from co-isolated fuscoatrol A[5] as proposed for the cytosporolides,[16] first via addition and cyclization to form **6**, an intermediate of the C-6 deacetylate of (+)-pestalotiopsin A,[1] and then the acid-catalyzed cyclizations of **6** via two intermediates **7** and **8** could afford the key putative intermediate **9**,[23] from which **1**–**3**, taedolidol,[3] 6-epitaedolidol,[3] and punctaporonin C (**4**)[7–9] could be generated via different routes (Scheme 1).
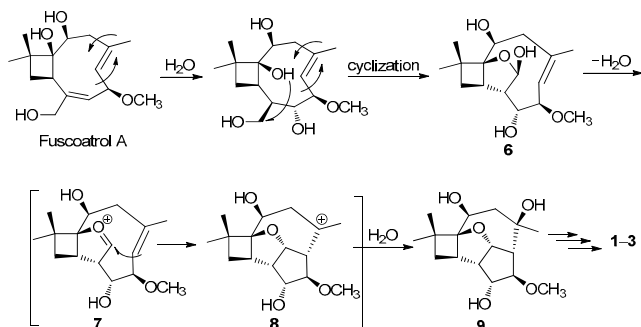
Scheme 1. Hypothetical biosynthetic pathways for compounds **1**–**3**

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. CD spectra were recorded on a JASCO J-815 spectropolarimeter. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. ¹H and ¹³C NMR data were acquired with Varian Mercury-400 and -500 spectrometers using solvent signals (acetone-d_6: δ_H 2.05/δ_C 29.8, 206.1; pyridine-d_5: δ_H 7.21, 7.58, 8.73; CDCl₃: δ_H 7.26/δ_C 76.7) as references. The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS data were recorded on a Bruker Esquire 3000^{plus} spectrometer, and HRESIMS data were obtained using Bruker APEX III 7.0 T and APEX II FT-ICR spectrometers, respectively.

Fungal Material. The culture of *Cytospora* sp. was isolated by Yang Hao from a soil sample collected on the Qinghai-Tibetan plateau at an altitude above 3,200 m, Linzhi, Tibet, China, in April, 2007. The isolation and identification of the strain have been previously described.[16a] The fungus was cultured on slants of PDA at 25 °C for 10 days. Agar plugs were cut into small pieces (about $0.5 \times 0.5 \times 0.5$ cm³) under aseptic conditions, 15 pieces were used to inoculate in three Erlenmeyer flasks (250 mL), each containing 50 mL of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract); the final pH of the media was adjusted to 6.5 and sterilized by autoclave. Three flasks of the inoculated media were incubated at 25 °C on a rotary shaker at 170 rpm for five days to prepare the seed culture. Spore inoculum was prepared by suspending the seed culture in sterile, distilled H₂O to give a final spore/cell suspension of 1×10^6/mL determined by microscope and hemocytometer. Fermentation was carried out in 12 Fernbach flasks (500 mL) each containing 80 g of rice. Distilled H₂O (120 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 psi 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 material was extracted with EtOAc (4×1.0 L), and the organic solvent was evaporated to dryness under vacuum to afford a crude extract (6.0 g). The extract was fractionated by silica gel VLC using petroleum ether–EtOAc gradient elution. The fraction (150 mg) eluted with 50% EtOAc was separated by Sephadex LH-20 column chromatography (CC) eluting with 1:1 CH₂Cl₂–MeOH. The resulting subfractions were combined and further purified by semipreparative RP HPLC (Agilent Zorbax SB-C₁₈ column; 5 μm; 9.4×250 mm; 2 mL/min) to afford cytosporinols A (**1**; 6.4 mg; t_R 19.93 min; 45% CH₃CN in H₂O for 25 min) and B (**2**; 2.0 mg, t_R 17.00 min; 25% CH₃CN in H₂O for 20 min). The fraction (300 mg) eluted with 30% EtOAc was eluted again through Sephadex LH-20 CC (1:1 CH₂Cl₂–MeOH) to afford cytosporinol C (**3**; 60.0 mg).

Cytosporinol A (1): colorless oil; $[\alpha]_D^{25}$ +86 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 208 (3.36) nm; IR (neat) ν_{max} 3424 (br), 2933, 2866, 1709, 1446, 1230, 1094, 1064, 1054, 1011, 975, 949 cm⁻¹; ¹H, ¹³C NMR, and HMBC data see Table 1; positive ion ESIMS *m/z* 303 [M + Na]⁺; HRESIMS *m/z* 303.1564 (calcd for C₁₆H₂₄O₄Na, 303.1567).

Preparation of (*R*)-(1a) and (*S*)-MTPA (1b) Esters.[19] A sample of **1** (2.0 mg, 0.007 mmol), (*S*)-MTPA Cl (3.0 μL, 0.017 mmol), and pyridine-d_5 (0.5 mL) were allowed to react in an NMR tube at ambient temperature for 24 h, with the ¹H NMR data of the *R*-MTPA ester derivative (**1a**) were obtained directly on the reaction mixture: ¹H NMR (pyridine-d_5, 400 MHz) δ 5.54 (1H, d, *J* = 4.0 Hz, H-12), 5.51 (1H, d, *J* = 8.0 Hz, H-11), 4.32 (1H, s, H-10), 4.17 (1H, dd, *J* = 12, 4.0 Hz, H-6), 3.70 (1H, t, *J* = 12 Hz, H-7a), 3.23 (3H, s, Me-16), 2.90 (1H, td, *J* = 8.0, 2.5 Hz, H-2), 2.62 (1H, ddd, *J* = 8.0, 4.0, 2.5 Hz, H-1), 2.16 (1H, dd, *J* = 12, 4.0 Hz, H-7b), 1.91 (1H, dd, *J* = 12, 8.0 Hz, H-3a), 1.74 (3H, s, Me-15), 1.57 (3H, s, Me-13), 1.54 (1H, dd, *J* = 12, 8.0 Hz, H-3b), 1.32 (3H, s, Me-14).

Similarly, the reaction mixture from another sample of **1** (2.0 mg, 0.007 mmol), (*R*)-MTPA Cl (3.0 μL, 0.017 mmol), and pyridine-d_5 (0.5 mL) was processed as described above for **1a** to afford **1b**: ^{1}H NMR (pyridine-d_5, 400 MHz) δ 5.56 (1H, d, *J* = 4.0 Hz, H-12), 5.55 (1H, d, *J* = 8.0 Hz, H-11), 4.26 (1H, dd, *J* = 12, 4.0 Hz, H-6), 4.19 (1H, s, H-10), 3.70 (1H, t, *J* = 12 Hz, H-7a), 3.23 (3H, s, ME-16), 2.92 (1H, td, *J* = 8.0, 2.5 Hz, H-2), 2.91 (1H, ddd, *J* = 8.0, 4.0, 2.5 Hz, H-1), 2.15 (1H, dd, *J* = 12, 4.0 Hz, H-7b), 2.00 (1H, m, H-3a), 2.00 (1H, m, H-3b), 1.65 (3H, s, Me-15), 1.61 (3H, s, Me-13), 1.35 (3H, s, Me-14).

Cytosporinol B (2): colorless oil; $[\alpha]_D^{25}$ −2.8 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log *ε*) 208 (3.38) nm; IR (neat) ν_{max} 3423 (br), 2936, 1718, 1445, 1382, 1236, 1104, 1043, 968 cm^{-1}; ^{1}H, ^{13}C NMR, and HMBC data see Table 2; positive ion ESIMS *m/z* 303 [M + Na]$^+$; HRESIMS *m/z* 303.1565 (calcd for $C_{16}H_{24}O_4Na$, 303.1567).

Cytosporinol C (3): colorless needles (MeOH–H$_2$O), mp 149–152 °C; $[\alpha]_D^{25}$ −8.8 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log *ε*) 209 (3.38) nm; IR (neat) ν_{max} 3473, 3444, 2960, 2930, 2874, 1731, 1466, 1401, 1385, 1235, 1150, 1133, 1118, 1093, 1067, 1038, 1000, 901 cm^{-1}; ^{1}H, ^{13}C NMR, HMBC, and NOESY data see Table 2; positive ion ESIMS *m/z* 377 [M + Na]$^+$; HRESIMS *m/z* 377.2300 (calcd for $C_{20}H_{34}O_5Na$, 377.2298).

X-ray Crystallographic Analysis of 3.[25] Upon crystallization from MeOH–H$_2$O (10:1) using the vapor diffusion method, colorless crystals were obtained for **3**. A crystal (0.40 × 0.30 × 0.08 mm) was separated from the sample and mounted on a glass fiber, and data were collected using a Rigaku Saturn CCD area detector with graphite-monochromated Mo Kα radiation, λ = 0.71073 Å at 173(2) K. Crystal data: $C_{20}H_{34}O_5$, *M* = 354.47, space group orthorhombic, $P2_12_12_1$; unit cell dimensions *a* = 5.8938 (17) Å, *b* = 13.939 (4) Å, *c* = 23.127 (7) Å, *V* = 1900.0 (10) Å^3, *Z* = 4, D_{calcd} = 1.239 mg/m^3, *μ* = 0.087 mm^{-1}, *F*(000) = 776. The structure was solved by direct methods using SHELXL-97[26] and refined 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 applied with the Siemens Area Detector Absorption Program (SADABS).[27] The 12519 measurements yielded 2026 independent reflections after equivalent data were averaged, and Lorentz and polarization corrections were applied. The final refinement gave R_1 = 0.0493 and wR_2 = 0.0990 [*I* > 2σ(*I*)].

Absolute Configuration of the 6,8-Diol Moiety in 3.[20] HPLC grade DMSO was dried with 4 Å molecular sieves. According to a published procedure, a mixture of 1:2 diol/Mo$_2$(OAc)$_4$ for **3** was subjected to CD measurements at a concentration of 0.6 mg/mL. The first CD spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary (about 10 min after mixing). The inherent CD was subtracted. The observed sign of the diagnostic band at around 400 nm in the induced CD spectrum was correlated to the absolute configuration of the 6,8-diol

moiety.

MTS Assay.[28] The assay was run in triplicate. In a 96-well plate, each well was plated with 2–5 × 10^3 cells (depending on the cell multiplication rate). After cell attachment overnight, the medium was removed, and each well was treated with 50 μL medium containing 0.1% DMSO, or appropriate concentrations of the test compounds and the positive control cisplatin (100 mM 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). The plate was incubated for 72 h at 37 °C in a humidified, 5% CO$_2$ atmosphere. Proliferation assessed by adding 20 μL of MTS (Promega) to each well in the dark, followed by a 90 min incubation at 37 °C. The assay plate was read at 490 nm using a microplate reader.

Electronic Supplementary Material

Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s13659-012-0018-z and is accessible for authorized users.

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[25] Crystallographic data for compound **3** have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 790902). Copies of the data can be obtained, free of charge, on application to the director, CCDC 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or email: deposit@ccdc.cam.ac.uk).

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