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Cytosporolides A−C, Antimicrobial Meroterpenoids with a Unique Peroxylactone Skeleton from *Cytospora* sp.

Yan Li,[†,‡] Subin Niu,[†] Bingda Sun,[†] Shuchun Liu,[†] Xingzhong Liu,[†] and Yongsheng Che*[,†]

Key Laboratory of Systematic Mycology & Lichenology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100190, People's Republic of China, and Graduate School of Chinese Academy of Sciences, Beijing 100039, People's Republic of China

cheys@im.ac.cn

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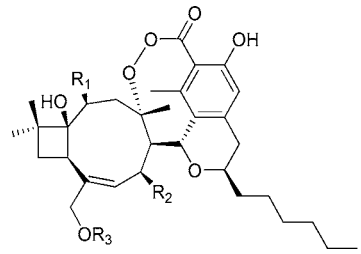
ABSTRACT



1 R_1 = OH R_2 = OCH_3 R_3 = H
2 R_1 = H R_2 = OH R_3 = H
3 R_1 = OH R_2 = OCH_3 R_3 = Ac

Cytosporolides A−C (1−3), three caryophyllene-derived meroterpenoids with a unique peroxylactone skeleton, were isolated from cultures of the fungus *Cytospora* sp. Their structures were elucidated by NMR spectroscopy, and the absolute configuration of the 5,6-diol moiety in 1 was assigned using Snatzke's method. Compounds 1−3 showed significant antimicrobial activity against the Gram-positive bacteria *Staphylococcus aureus* and *Streptococcus pneumoniae*.

Caryophyllenes have been encountered frequently in fungal natural products. Examples include fuscoatrol A (**4**), an antimicrobial and cytotoxic agent isolated from the marine fungus *Humicola fuscoatra* KMM 4629;[1] the punctaporonins from the fungus *Poronia punctata*;[2−7] the pestalotiopsins, 6-hydroxpunctaporonins A, B, and E, taedolidol, and 6-epi-taedolidol from *Pestalotiopsis* spp.;[8−11] and Sch 725432, 601253, 601254, and 725434 from *Chrysosporium pilosum*.[12]

[†] Institute of Microbiology.
[‡] Graduate School of Chinese Academy of Sciences.

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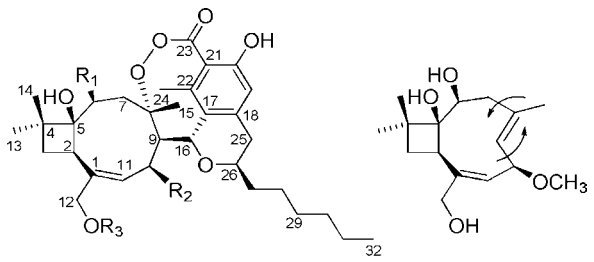
Some unique caryophyllenes have also been isolated from the plants, such as guajadial and psidials A−C from the leaves of *Psidium guajava* L[13,14] and lychnopholic acid from *Lychnophora affinis* Gardn.[15] On the other hand, natural peroxylactones are very rare; only three examples have been reported to date, with two isolated from the brown seaweed *Taonia atomaria*[16] and plakinidone from the caribbean sponge *Plakortis angulospiculatus*,[17] respectively.

Chemical studies of *Cytospora* spp. have afforded a variety of bioactive natural products.[18−21] In a search for new antimicrobial agents from fungi inhabiting unique environments, we initiated a chemical investigation of the fungus *Cytospora* sp. isolated from a soil sample that was collected on the Qinghai-Tibetan plateau at an altitude above 3,200 m. The strain was grown in a solid-substrate fermentation culture. Its EtOAc extract showed activity against the Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538) and *Streptococcus pneumoniae* (CGMCC 1.1692). Bioassay-directed fractionation of the extract afforded cytosporolides A−C (**1**−**3**), three caryophyllene-derived meroterpenoids featuring a novel peroxylactone skeleton as the active principles. In addition, fuscoatrol A (**4**),[1] a putative precursor of **1**−**3**, was also isolated. Details of the structure assignment and plausible biogenesis of **1**−**3** are reported herein.



Fuscoatrol A (**4**) was isolated as the major component (50.0 mg), and its structure was readily identified by comparison of its NMR and MS data with those reported.[1] Compound **4** exists as two slowly equilibrating atropisomers ($\alpha\alpha$:$\alpha\beta$ in a 3:1 ratio) in chloroform at room temperature. Its relative configuration was assigned by X-ray crystallography.[1]

Compound **1** was assigned a molecular formula of $C_{33}H_{48}O_9$ (10 degrees of unsaturation) by HRESIMS (*m*/*z* 611.3196 $[M + Na]^+$). Its NMR spectroscopic data (Table 1) revealed the presence of three exchangeable protons, six methyls (one methoxyl), nine methylenes, one oxymethine, eight aromatic/olefinic carbons (two of which are protonated), three sp^3 quaternary carbons (two oxygenated), and one carboxylic carbon (δ_C 171.9). These data accounted for all of the NMR resonances except for one exchangeable proton. The $^1H-^1H$ COSY NMR data of **1** showed the four isolated spin systems of C-2−C-3, C-6−C-7, C-9−C-11 (including C-16), and C-25−C-32. HMBC correlations from H_3-13 and H_3-14 to C-3, C-4, and C-5; from H_2-3 to C-2, C-4, C-5, C-13, and C-14; and from H-2 to C-3 and C-5 completed the same cyclobutane ring with the two C-4 attached germinal methyl groups as found in **4**.[1] Correlations from H-6 and H_2-7 to C-5 led to the connection of C-5 to C-6. In turn, correlations from H_3-15 to C-7, C-8, and C-9 and from the *O*-methyl protons OCH_3-10 to C-10 indicated that C-7, C-9, and C-15 are all attached to C-8 and the *O*-methyl group is connected to C-10, while those from H_2-12 to C-1, C-2, and C-11 indicated that C-2 and C-11 are all allylic to the C-1/C-11 olefin, completing a nine-membered ring fused to the cyclobutane unit at C-2 and C-5, similar to that found in **4**. Comparing the chemical shifts of the oxygented sp^3 carbons C-5 (δ_C 81.9), C-6 (δ_C 70.3), and C-12 (δ_C 66.2) with those of **4** indicated that the three carbons all bear a hydroxyl group. Therefore, a bicyclo[7.2.0]undeca-1-ene-5,6-diol partial structure was established for **1**, which is nearly identical to **4**, except for the difference in the C-8/C-9 olefin unit.

The remaining six sp^2 carbons (one protonated) in **1** suggested the presence of an aryl moiety, a deduction supported by relevant HMBC data. HMBC correlations from H_3-24 to C-22 located this methyl group at C-22, while those from H_2-25 to C-17, C-18, and C-19 led to the connection of C-25 to C-18. HMBC cross-peaks from H-16 to C-17, C-18, and C-22 revealed the connection of C-16 to C-17. A key correlation from H-26 to C-16 established a dihydropyran moiety fused to the aryl ring at C-17/C-18, completing the isochroman partial structure. HMBC correlations from the phenolic proton (OH-20) to C-19, C-20, and C-21 (in C_6D_6) indicated that C-20 bears a hydroxyl group. A four-bond *W*-type coupling from H-19 to C-23 enabled the connectivity between the C-23 carboxylic carbon and C-21,[22] a deduction supported by the downfield shift of OH-20 due to formation of an intramolecular hydrogen bond. The chemical shift of the oxygenated carbon C-8 (δ_C 87.5) was significantly downfield compared to the known 6-hydroxypunctaporonin B (δ_C 74.2);[11] this observation and the unsaturation requirement for **1** required a peroxide bond between C-8 and C-23. Collectively, these data allowed assignment of the planar structure of **1**.

The relative configuration of **1** was determined on the basis of NOESY data (Figure 1). The C-1/C-11 olefin was assigned

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Table 1. NMR Spectroscopic Data for **1** in Acetone-d_6

position	δ_H[a] (J in Hz)	δ_C[b]	HMBC (H → C#)
1		138.0	
2	3.20, dd (10.6, 8.4)	39.3	1, 3, 5, 11, 12
3a	2.19, t (10.6)	34.2	1, 2, 4, 5, 13, 14
3b	1.55, dd (10.6, 8.4)		2, 5, 13
4		41.0	
5		81.9	
6	4.03, d (4.7)	70.3	2, 5, 7, 8
7a	3.08, dd (16.5, 4.7)	46.2	5, 6, 8
7b	1.71, d (16.5)		5, 6, 8, 9, 15
8		87.5	
9	2.60, dd (9.7, 4.3)	49.6	7, 8, 15, 16
10	4.55, d (4.3)	77.4	1, 8, 9, 11, OCH_3-10
11	5.83, s	131.5	1, 2, 5, 9, 12
12a	4.29, d (12.6)	66.2	1, 2, 11
12b	4.06, (12.6)		1, 2, 11
13	1.18, s	24.5	3, 4, 5, 14
14	1.07, s	23.9	3, 4, 5, 13
15	1.42, s	25.4	7, 8, 9
16	5.02, d (9.7)	63.0	9, 10, 17, 18, 22, 26
17		113.5	
18		145.3	
19	6.48, s	109.4	17, 20, 21, 23, 25
20		163.8	
21		99.2	
22		151.0	
23		171.9	
24	1.28, br s	26.4	22
25a	2.95, dd (15.6, 4.7)	34.9	17, 18, 19, 26, 27
25b	2.65, dd (15.6, 9.3)		17, 18, 19, 26, 27
26	3.82, m	72.7	16, 18
27a	1.79, m	36.6	
27b	1.64, m		29
28	1.29, m	29.3	
29	1.29, m	29.6	
30	1.33, m	32.6	
31	1.30, m	23.3	30, 32
32	0.87, t (6.9)	14.3	30, 31
OH-5	4.63, br s		
OH-6	4.69, br s		
OH-20	12.00, br s		19, 20, 21[c]
OCH_3-10	3.47, s	59.2	10

[a] Recorded at 500 MHz. [b] Recorded at 125 MHz. [c] Recorded in C_6D_6 at 500 MHz.

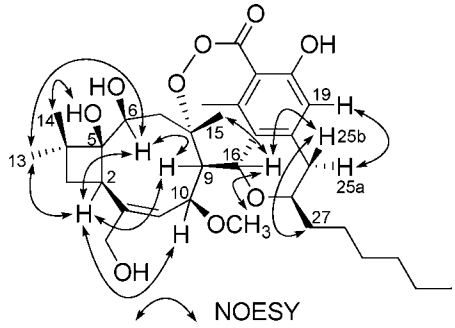


Figure 1. Key NOESY correlations for **1**.

E-geometry by correlations of H-11 with H-12b and OCH_3-10. NOESY cross-peaks of H-2 with H-6, H-9, H-10, and H_3-13, and of H-6 with H-9 and H_3-13 placed these protons on the same face of the ring system. While those of OH-5 with H_3-14, H-16 with H_3-15, OCH_3-10, and of H-25b, and of H-25b with H_2-27 were used to place them on the opposite face. On the basis of these data, the relative configuration of **1** was established as shown.

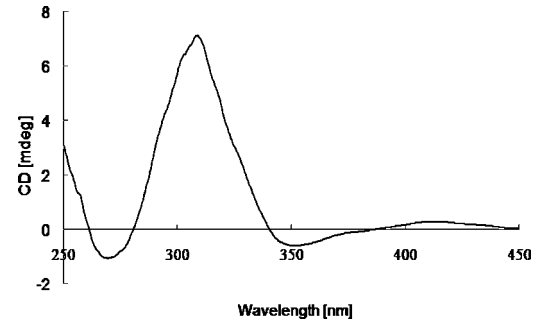


Figure 2. CD spectrum of **1** in DMSO containing $Mo_2(OAc)_4$ with the inherent CD spectrum subtracted.

The absolute configuration of the 5,6-diol moiety in **1** was assigned using the in situ dimolybdenum CD method developed by Frelek.[23,24] Upon addition $Mo_2(OAc)_4$ to a solution of **1** in DMSO, a metal complex was generated as an auxiliary chromophore. Since the contribution from the inherent CD resulting from the C-23 carbonyl group was subtracted to give the induced CD of the complex, the observed sign of the Cotton effect in the induced spectrum originates solely from the chirality of the *vic*-diol moiety expressed by the sign of the O−C−C−O torsion angle. The positive Cotton effects observed at 306 and 410 nm, respectively, in the induced CD spectrum (Figure 2) permitted assignment of the 5*S* and 6*S* configuration on the basis of the empirical rule proposed by Snatzke,[25] with the bulkier cyclobutane group pointing away from the remaining portion of the complex (Figure 3). Combining the relative configuration established by NOESY data, the 2*R*, 5*S*, 6*S*, 8*S*, 9*R*, 10*S*, 16*R*, and 26*R* configuration was assigned for **1**.

Compound **2** gave a pseudomolecular ion $[M - H]^-$ peak at *m*/*z* 557.3137 by HRESIMS, consistent with the molecular formula $C_{32}H_{46}O_8$ (10 degrees of C═C unsaturation). Analy-

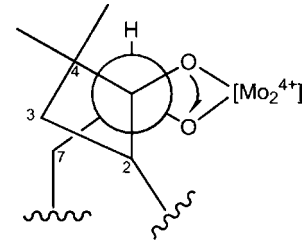
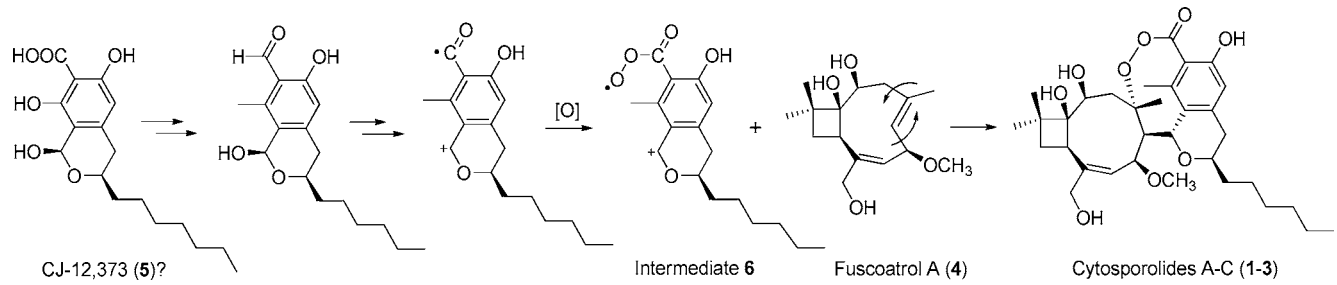


Figure 3. Conformation of the Mo_2^{4+} complex of **1**.

Scheme 1. Hypothetical Biosynthetic Pathways for **1**−**3**



sis of its NMR spectroscopic data revealed structural features similar to those of **1**, except that the C-6 oxymethine (δ_H/δ_C 4.03/70.3) was replaced by a methylene (δ_H/δ_C 1.27, 1.60/28.3) and the resonances of the C-10 *O*-methyl group were absent in **2**. These observations were confirmed by relevant $^1H-^1H$ COSY correlations, completing the planar structure of **2**. The absolute configuration of **2** was deduced by analogy to **1**.

The elemental composition of **3** was determined to be $C_{35}H_{50}O_{10}$ (11 degrees of C═C unsaturation) by HRESIMS (*m*/*z* 629.3312 $[M - H]^-$). The ^1H and ^{13}C NMR spectra of **3** showed resonances nearly identical to those of **1**, except that the oxygenated methylene protons (H_2-12) were shifted downfield (δ_H 4.29 and δ_H 4.06 in **1**; δ_H 5.02 and δ_H 4.93 in **3**). In addition, NMR resonances corresponding to an acetyl group (δ_H 2.02; δ_C 23.5 and 173.6) were observed, indicating that the oxygen atom attached to C-12 was acylated, which was confirmed by an HMBC correlation from H_2-12 to the carboxylic carbon at δ_C 173.6. Since **3** is an acetate of **1** at C-12, its absolute configuration was presumably the same as that of **1**.

Cytosporolides A−C (**1**−**3**) were tested for activity against the Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538) and *Streptococcus pneumoniae* (CGMCC 1.1692) (Table 2). Compound **3** is the most potent one among these metabolites, with IC_{50} values of 1.98 and 1.16 μg/mL, respectively, while the positive control antimicrobial peptide (AMP) showed IC_{50} values of 0.021 and 0.38 μg/mL, respectively, against the two pathogens.

Table 2. Antibacterial Effects of Compounds **1**−**3**

	IC_{50} (μg/mL)	
compound	*S. aureus*	*S. pneumoniae*
1	8.42 ± 0.10	10.72 ± 0.73
2	14.26 ± 0.57	>20.0
3	1.98 ± 0.04	1.16 ± 0.02
AMP	0.021 ± 0.003	0.38 ± 0.03

Biogenetically, **1**−**3** could be derived from the coisolated known compound **4**,[1] and a putative biosyntheitc intermediate **6** (Scheme 1);[16] whereas **6** could be derived from the isochroman type of precursors, such as CJ-12,373 (**5**), a metabolite isolated from *Penicillium* sp. CL22557.[26] Even though **5** and/or related compounds were not isolated from the same extract in the current study, **1** could be generated via a series of reactions from **5** (or its analogues) and **4** as illustrated in the hypothetical biosynthetic pathways shown in Scheme 1.

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Supporting Information Available: Experimental procedures, characterization data, and ^1H and ^{13}C NMR spectra of **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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