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Isolation and Characterization of Aphidicolin and Chlamydosporol Derivatives from *Tolypocladium inflatum*

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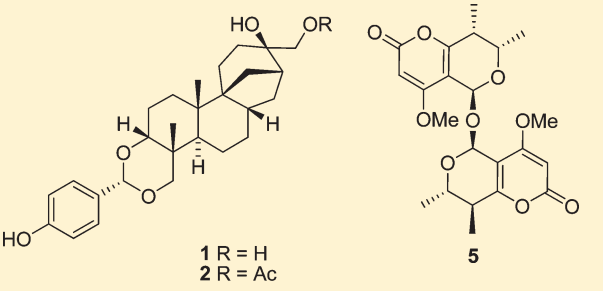
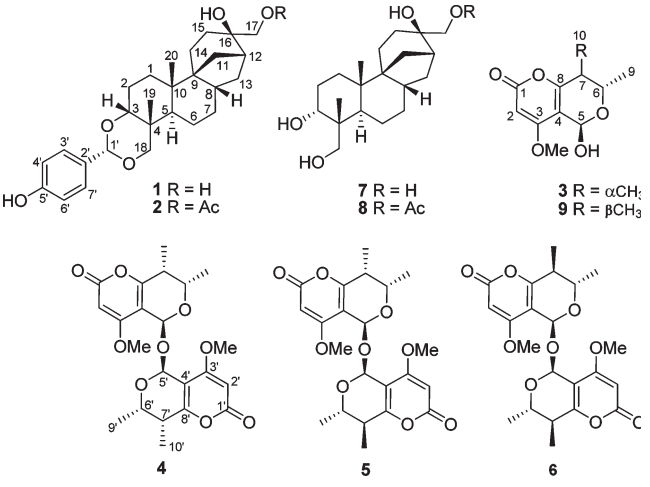
Supporting Information

ABSTRACT: Six new secondary metabolites including two aphidicolin analogues, inflatins A (**1**) and B (**2**), and four chlamydosporol derivatives, inflatins C−F (**3**−**6**), have been isolated from the crude extract of *Tolypocladium inflatum*. The structures of **1**−**6** were determined mainly by NMR experiments, and **4** and **5** were further confirmed by X-ray crystallography. The absolute configurations of C-16 in **1** and C-5 in **3** were deduced via the circular dichroism data of the in situ formed $[Rh_2(OCOCF_3)_4]$ complexes, whereas that of **4** was assigned by X-ray crystallography using Cu Kα radiation. Compounds **1** and **2** showed modest cytotoxicity against a panel of eight human tumor cell lines.



Fungi are capable of producing a variety of bioactive secondary metabolites.[1] Since the secondary metabolism of fungi may be influenced by selection pressures exerted by other organisms and the environment in which they reside, those species thriving in unique and competitive niches are especially likely to produce bioactive natural products with diverse and interesting structural features.[2,3] On the basis of this consideration and the documented success in finding new bioactive natural products from special types of fungi,[4] we initiated chemical studies of the *Cordyceps*-colonizing fungi[5] and those that were isolated from the soil samples surrounding *Cordyceps sinensis*.[6] In the current work, the ascomycetous fungus *Tolypocladium inflatum* (SCK6-CP14) was isolated from a soil sample on the surface of *C. sinensis* collected in Kangding, Sichuan, People's Republic of China. An EtOAc extract prepared from a solid-substrate culture showed cytotoxicity against a panel of eight human tumor cell lines including A549 (human lung adenocarcinoma), CNE1-LMP1 (stable oncoprotein LMP1 integrated nasopharyngeal carcinoma), A375 (human malignant melanoma), MCF-7 (human breast cancer), MGC (human gastric cancer), EC109 (human esophageal cancer), PANC-1 (human pancreatic carcinoma), and Hep3B-2 (human hepatoma carcinoma). Fractionation of the extract led to the isolation of six new metabolites including two aphidicolin analogues, inflatins A (**1**) and B (**2**), and four chlamydosporol derivatives, inflatins C−F (**3**−**6**), together with three known ones, aphidicolin (**7**),[7,8] aphidicolin-17-monoacetate (**8**),[9] and isochlamydosporol (**9**).[10] Details of the isolation, structure elucidation, and cytotoxicity of these compounds are reported herein.



RESULTS AND DISCUSSION

Inflatin A (**1**) was assigned the molecular formula $C_{27}H_{38}O_5$ (nine degrees of unsaturation) on the basis of HRESIMS. Analysis of its ^1H and ^{13}C NMR data (Table 1) revealed three exchangeable protons (δ_H 3.07, 3.50, and 8.38, respectively), two methyl groups, 10 methylenes (two oxygenated), five methines

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Table 1. NMR Data for 1 and 2

	1			2		
pos.	δ_C[a], mult.	δ_H[b] (J in Hz)	HMBC[a]	δ_C[c], mult.	δ_H[d] (J in Hz)	HMBC[c]
1a	27.9, CH_2	2.20, td (11.0, 2.9)	9, 20	27.1, CH_2	2.16−2.23[e]	20
1b		0.95, dt (11.0, 2.5)	3, 5		0.97−1.04[e]	5
2a	27.8, CH_2	1.68−1.72[e]		26.9, CH_2	1.68−1.73[e]	
2b		1.30−1.38[e]			1.30−1.38,[e]	
3	81.7, CH	3.62, t (3.0)	1, 5, 19	81.3, CH	3.65, s	1, 18, 19
4	35.6, qC			35.1, qC		
5	34.6, CH	2.84, dd (10.5, 2.5)	1, 19, 20	33.8, CH	2.82, dd (10.5, 1.9)	19, 20
6a	23.2, CH_2	1.63, m		22.5, CH_2	1.59−1.66[e]	
6b		1.28−1.36[e]			1.21−1.28[e]	
7a	25.2, CH_2	1.92−2.02[e]	9	24.4, CH_2	2.06−2.10[e]	13
7b		1.68−1.70[e]	5, 9		1.67−1.73[e]	13
8	41.1, CH	1.96−2.08[e]	10	40.1, CH	1.98−2.08[e]	10
9	49.9, qC			49.1, qC		
10	40.6, qC			39.9, qC		
11a	33.4, CH_2	1.89−1.91[e]	8, 13, 14, 16	32.5, CH_2	1.82−1.87[e]	8, 13, 14, 16
11b		1.25−1.37[e]	14, 16		1.35−1.42[e]	14, 16
12	42.3, CH	2.13, t (5.7)	8, 9, 15, 17	41.7, CH	2.10−2.16[e]	8, 9, 15
13a	31.9, CH_2	1.70−1.72[e]	9, 16	31.5, CH_2	1.78−1.82[e]	9, 16
13b		0.99, dd (11.5, 7.0)	11, 16		0.94−0.99[e]	8, 11, 16
14a	25.5, CH_2	1.97−2.12[e]	16	24.5, CH_2	1.91−2.02[e]	10, 11, 16
14b		1.71−1.76[e]	16		1.67−1.73[e]	16
15a	29.2, CH_2	1.48, dt (12.0, 2.7)	17	28.4, CH_2	1.49−1.55[e]	
15b		1.30, td (12.0, 5.0)	9, 12		1.37−1.44[e]	9
16	74.4, qC			73.6, qC		
17a	68.3, CH_2	3.37, dd (9.0, 5.0)	12, 15	70.0, CH_2	4.05, d (11.0)	12, 15, 21
17b		3.27, dd (9.0, 5.0)	12, 15		3.99, d (11.0)	12, 15, 21
18a	76.2, CH_2	4.02, d (10.0)	3, 1′	75.9, CH_2	4.11, d (12.0)	3, 1′
18b		3.16, d (10.0)	3, 5, 19, 1′		3.21, d (12.0)	3, 5, 19, 1′
19	17.2, CH_3	0.76, s	3, 18	17.0, CH_3	0.80, s	3, 5, 18
20	16.0, CH_3	1.05, s	1, 5, 9	15.6, CH_3	1.07, s	1, 5, 9
21				171.3, qC		
22				15.6, CH_3	2.08, s	21
1′	102.5, CH	5.41, s	3, 18, 3′, 7′	101.7, CH	5.46, s	3, 18, 3′, 7′
2′	132.0, qC			131.7, qC		
3′	128.6, CH	7.33, d (7.0)	1′, 5′, 7′	127.9, CH	7.40, d (8.3)	1′, 5′, 7′
4′	115.5, CH	6.81, d (7.0)	2′, 6′	115.2, CH	6.82, d (8.3)	2′, 6′
5′	158.5, qC			156.1, qC		
6′	115.5, CH	6.81, d (7.0)	2′, 4′	115.2, CH	6.82, d (8.3)	2′, 4′
7′	128.6, CH	7.33, d (7.0)	1′, 3′, 5′	127.9, CH	7.40, d (8.3)	1′, 3′, 5′
OH-16		3.07, s				
OH-17		3.50, t (5.0)				
OH-5′		8.38, s				

[a] Recorded at 150 MHz in acetone-d_6. [b] Recorded at 600 MHz in acetone-d_6. [c] Recorded at 125 MHz in $CDCl_3$. [d] Recorded at 500 MHz in $CDCl_3$. [e] Signals overlapping.

(two oxygenated including one with double oxygenation at δ_C 102.5), six aromatic/olefinic carbons (four of which are protonated), and four sp^3 quaternary carbons (one oxygenated). These data accounted for all the NMR resonances for **1** and are consistent with the molecular formula $C_{27}H_{38}O_5$. NMR resonances for the four aromatic protons were observed as two sets of doublets (7.0 Hz each) at 6.81 and 7.33 ppm, respectively, suggesting the presence of a *p*-substituted phenyl ring. The remaining NMR resonances of **1** closely matched those of aphidicolin (**7**),[8] a known compound that was co-isolated as the major component from the crude extract, implying that **1** could be a condensation product of *p*-hydroxybenzaldehyde and **7**. These observations were supported by HMBC correlations from the acetal proton (H-1′; δ_H 5.41) to C-3, C-18, C-3′, and C-7′. On the basis of these data, the planar structure of **1** was established as shown.

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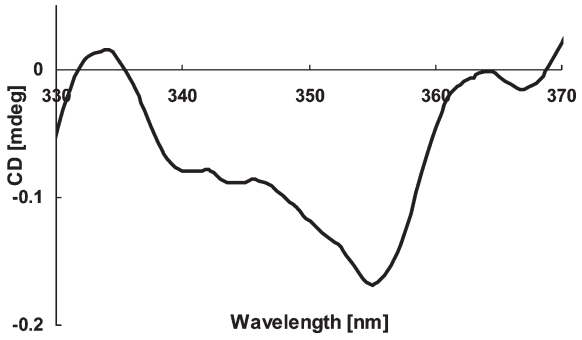

Figure 1. CD spectrum of the Rh complex of **1** with the inherent CD spectrum subtracted.

The relative configuration of **1** was determined on the basis of NOESY data and by comparison with that of aphidicolin (**7**).[7] Key NOESY correlations of H-1′ with H-3 and H-18b established the relative configuration of C-1′, whereas the remaining portion of **1** was deduced to have the same relative configuration as the known precedents **7** and **8**.[7−9]

The absolute configuration of the C-16 tertiary alcohol in **1** was determined via the CD data of the in situ formed $[Rh_2(OCOCF_3)_4]$ complex,[11] with the inherent contribution subtracted. Upon addition of $[Rh_2(OCOCF_3)_4]$ to a solution of **1** in CH_2Cl_2, a metal complex was generated as an auxiliary chromophore. It has been demonstrated that the sign of the E band (at ca. 350 nm) can be used to correlate the absolute configuration of a tertiary alcohol by applying the bulkiness rule.[11,12] In this experiment, the Rh complex of **1** displayed a negative E band (Figure 1), correlating to the 16*R* absolute configuration. This assignment is consistent with that reported for aphidicolin (**7**).[7] Therefore, the 1′*S* absolute configuration was assigned for **1**.

Inflatin B (**2**) gave a pseudomolecular ion $[M + H]^+$ peak, consistent with a molecular formula of $C_{29}H_{40}O_6$ (10 degrees of unsaturation). Analysis of its ^1H and ^{13}C NMR spectroscopic data (Table 1) revealed nearly identical structural features to those found in **1**, except that the oxygenated methylene protons (H_2-17) at 3.27 and 3.37 ppm were significantly downfield (δ_H 3.99 and 4.05, respectively). In addition, NMR resonances corresponding to an acetyl group (δ_H/δ_C 2.08/15.6, 171.3) were observed, indicating that the C-17 oxygen of **2** was acylated, which was supported by HMBC cross-peaks from H_2-17 to the carboxylic carbon at 171.3 ppm. On the basis of these data, **2** was determined as the C-17 monoacetate of **1**. The relative and absolute configurations of **2** were deduced as shown by analogy to **1**, which was also supported by the nearly identical CD spectra recorded for both compounds.

The elemental composition of inflatin C (**3**) was established as $C_{11}H_{14}O_5$ (five degrees of unsaturation) by HRESIMS, which is the same as the co-isolated known compound isochlamydosporol (**9**).[10] Comparison of their NMR data indicated that they differ only in the configuration at the stereogenic center C-7. A coupling constant of 2.0 Hz observed for H-6 in **3**, compared to 10.0 Hz for the same proton in **9**, indicated that H-6 and H-7 in **3** are all pseudoequatorially oriented with respect to the 3,6-dihydro-2*H*-pyran ring.[13] A NOED correlation of H-5 with H_3-10 revealed their proximity in space, thereby completing the relative configuration of **3**. Since the absolute configuration of compound **9** was not assigned,[10] the configuration of the C-5 secondary alcohol in both **3** and **9** was individually determined to be *R* using the CD data of the in situ generated $[Rh_2(OCOCF_3)_4]$ complexes (Figures S20 and S21; Supporting Information). Therefore, the 5*R*, 6*S*, and 7*R* and 5*R*, 6*S*, and 7*S* absolute configurations were proposed for **3** and **9**, respectively.

Inflatin D (**4**) gave a pseudomolecular ion $[M + H]^+$ peak by HRESIMS, consistent with a molecular formula of $C_{22}H_{26}O_9$ (10 degrees of unsaturation). Even though its NMR spectroscopic data (Table 2) closely resembled those of **3**, the NMR spectra of **4** showed only half of the resonances required by the elemental composition, indicating that **4** is a homodimer of **3**. Key HMBC correlations from H-5 to C-5′ and from H-5′ to C-5 connected the two units of **3** via an ether linkage to complete the gross structure of **4**.

The relative configuration of **4** was deduced by analogy to **3** and was further confirmed by single-crystal X-ray crystallography (Figure 2). The presence of a relatively high percentage of oxygen in **4** exhibited enough anomalous dispersion of Cu Kα radiation to allow assignment of its absolute configuration.[14] Therefore, the absolute configuration of **4** was initially proposed as shown on the basis of the value of the Flack parameter 0.2(2),[15] which was subsequently confirmed by comparison of its CD spectrum with that of **3** (Figures S15 and S16; Supporting Information).

Inflatin E (**5**) was assigned the same molecular formula $C_{22}H_{26}O_9$ as **4** by HRESIMS. Its NMR spectroscopic data (Table 2) showed structural characteristics of both **3** and **9**,[10] suggesting that **5** could be a heterodimer derived from the two compounds. This postulation was supported by an HMBC correlation from H-5 to C-5′ (δ_C 87.9) and was confirmed by X-ray crystallography (Figure 3). Since precursors **3** and **9** were both isolated in the current work, the absolute configuration of **5** was deduced as shown on the basis of biogenetic considerations.

Inflatin F (**6**) was obtained as a white powder. HRESIMS data for **6** gave the same molecular formula $C_{22}H_{26}O_9$ as **5**. Its NMR data (Table 2) are nearly identical to those of **9**[10] and showed only half of the resonances of the molecular formula, indicating that **6** is a homodimer of **9**. This observation was supported by relevant HMBC correlations. The absolute configuration of **6** was deduced by analogy to **9**.

To verify that the new metabolites **4**−**6** are authentic natural products, a portion of the freeze-dried fermented rice substrate was extracted with distilled, HPLC-grade EtOAc, and the resulting extract was subjected to RP HPLC analysis using distilled, HPLC-grade H_2O and MeOH as solvents. Compounds **4**−**6** were identified on the HPLC chromatogram of the crude extract by comparison of their retention times with the pure compounds, indicating that **4**−**6** are indeed naturally occurring metabolites.

Compounds **1**−**6** were tested against a panel of eight human tumor cell lines (Table 3). Compounds **1** and **2** showed the most potent cytotoxic effects against Hep3B-2 cells, with IC_{50} values of 14.8 and 7.3 μM, respectively, while the positive control paclitaxel showed an IC_{50} value of 1.6×10^{-5} μM. However, only **6** of the dimeric isochlamydosporols displayed modest cytotoxicity against Hep3B-2 cells, with an IC_{50} value of 57.5 μM. Compounds **1** and **2** also showed cytotoxicity against the negative control HaCaT (human keratinocyte) cells, implying the lack of selectivity for these metabolites. Due to assay limitations, compounds **1** and **2** were not evaluated for DNA polymerase inhibition or antiviral activity.

Aphidicolin was originally isolated from *Cephalosporium aphidicola* as a DNA polymerase α inhibitor[7−9] and has been the subject of extensive studies including total synthesis[16] and

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

	4			5			6		
pos.	δ_C, mult.	δ_H (J in Hz)	HMBC	δ_C, mult.	δ_H (J in Hz)	HMBC	δ_C, mult.	δ_H (J in Hz)	HMBC
1	163.1, qC			163.1, qC			162.9, qC		
2	88.6, CH	5.47, s	4	88.7, CH	5.50, s	4	88.3, CH	5.49, s	4
3	169.2, qC			169.1, qC			169.0, qC		
4	107.3, qC			107.3, qC			107.8, qC		
5	88.1, CH	5.90, s	6, 8, 5′	88.2, CH	5.91, s	6, 8, 5′	88.0, CH	5.92, s	6, 8, 5′
6	66.2, CH	4.59, qd (6.5, 3.5)		66.2, CH	4.61, qd (6.5, 3.2)		69.9, CH	4.14, dq (10.5, 5.5)	
7	36.2, CH	2.47, qd (7.0, 3.5)		36.2, CH	2.50, qd (7.0, 3.2)		38.7, CH	2.53, dq (10.5, 6.0)	4, 9
8	165.4, qC			165.4, qC			163.4, qC		
9	17.2, CH_3	1.32, d (6.5)	7	17.2, CH_3	1.36, d (6.5)	7	19.2, CH_3	1.42, d (5.5)	7,
10	11.5, CH_3	1.14, d (7.0)	6, 8	11.5, CH_3	1.17, d (7.0)	6, 8	12.9, CH_3	1.20, d (6.0)	6, 8
11	56.9, CH_3	3.90, s	3	56.9, CH_3	3.95, s	3	56.8, CH_3	3.94, s	3
1′	163.1, qC			162.9, qC			162.9, qC		
2′	88.6, CH	5.47, s	4′	88.3, CH	5.51, s	4′	88.3, CH	5.49, s	4′
3′	169.2, qC			169.1, qC			169.0, qC		
4′	107.3, qC			107.9, qC			107.8, qC		
5′	88.1, CH	5.90, s	6′, 8′, 5	87.9, CH	5.96, s	6′, 8′, 5	88.0, CH	5.92, s	6′, 8′, 5
6′	66.2, CH	4.59, qd (6.5, 3.5)		69.9, CH	4.18, dq (10.0, 6.0)		69.9, CH	4.14, dq (10.5, 5.5)	
7′	36.2, CH	2.47, qd (7.0, 3.5)		38.7, CH	2.56, dq (10.0, 7.0)		38.7, CH	2.53, dq (10.5, 6.0)	4′, 9′
8′	165.4, qC			163.4, qC			163.4, qC		
9′	17.2, CH_3	1.32, d (6.5)	7′	19.2, CH_3	1.43, d (6.0)	7′	19.2, CH_3	1.42, d (5.5)	7′
10′	11.5, CH_3	1.14, d (7.0)	6′, 8′	12.9, CH_3	1.22, d (7.0)	6′, 8′	12.9, CH_3	1.20, d (6.0)	6′, 8′
11′	56.9, CH_3	3.90, s	3′	56.8, CH_3	3.95, s	3′	56.8, CH_3	3.94, s	3′

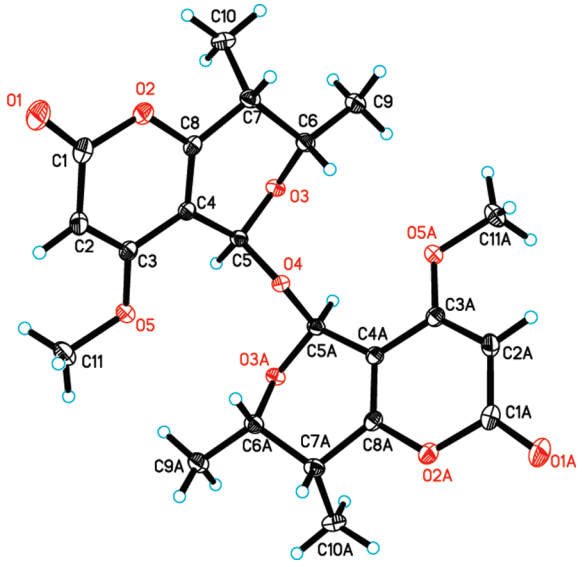

Figure 2. Thermal ellipsoid representation of 4. (Note: A different numbering system is used for the structural data deposited with the CCDC.)

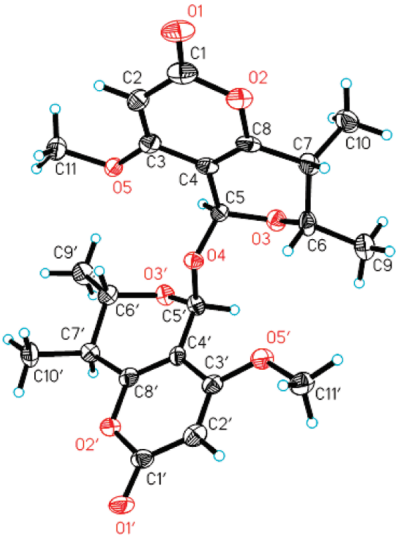

Figure 3. Thermal ellipsoid representation of 5.

biosynthesis[17] due to its significant antitumor and antiviral activity.[18] The aphidicolins are unique tetracyclic diterpenoids featuring the bicyclo[3.2.1]octane subunit. Other examples belonging to this class of natural products include aphidicolanoic acid and aphidicolanepentol isolated from *C. aphidicola*;[19] aphidicolanediol, aphidicolanol, aphidicolene, and 3-deoxyaphidicolin from *Phoma betae*;[20] maritimol, maristeminol, stemodanol, stemodanediol, stemodin, stemodinoside, and stemodinone from *Stemodia* sp.;[21] and scopadulin from *Scoparia dulcis*.[22] Inflatins A (**1**) and B (**2**) are new analogues of aphidicolin derivatives possessing a 1,3-dioxane moiety *cis* fused to the aphidicolin core at C-3/C-4, like that found in the natural precedent aphidicolin-3,18-orthoacetate.[20] Structurally, inflatin A (**1**) is likely derived from an aphidicolin and a *p*-hydroxybenzaldehyde moiety through formation of an acetal between C-18 and C-1′, whereas inflatin B (**2**) is the C-17 acetate of **1**. Inflatin C (**3**) is a C-7 stereoisomer

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Table 3. Cytotoxicity of Compounds 1−6 against Human Tumor Cell Lines

	IC_{50} (μM)								
compound	A549[a]	CNE1-LMP1[b]	A375[c]	MCF-7[d]	MGC[e]	EC109[f]	PANC-1[g]	Hep3B-2[h]	HaCaT[i]
1	75.3	39.1	47.3	33.5	54.5	47.1	19.9	14.8	49.1
2	25.0	15.0	25.5	75.2	>100	70.8	53.2	7.3	55.1
3	>100	>100	>100	>100	>100	>100	>100	>100	>100
4	>100	>100	>100	>100	>100	>100	>100	>100	>100
5	>100	>100	>100	>100	>100	>100	>100	>100	>100
6	>100	>100	>100	>100	>100	>100	>100	57.5	>100
paclitaxel	3.0×10^{-2}	4.2×10^{-3}	8.9×10^{-3}	1.4×10^{-2}	6.4×10^{-3}	0.14	1.1×10^{-3}	1.6×10^{-5}	0.024

[a] Lung adenocarcinoma cells. [b] Stable oncoprotein LMP1 integrated nasopharyngeal carcinoma cells. [c] Malignant melanoma cells. [d] Breast cancer cells. [e] Gastric cancer cells. [f] Esophageal cancer cells. [g] Pancreatic carcinoma cells. [h] Hepatoma carcinoma cells. [i] Keratinocyte cells.

of **9**,[10] possessing the 7,8-dihydropyrano[4,3-*b*]pyran-2(5*H*)-one moiety. Other natural products incorporating the 7,8-dihydropyrano[4,3-*b*]pyran-2(5*H*)-one unit include coarctatin from *Chaetomium coarctatum*,[23] multiforisin C from *Gelasinospora multiforis*,[24] and neovasinin and neovasinone from *Neocosmospora vasinfecta*.[25] Inflatins D (**4**) and F (**6**) are homodimers of **3** and **9**, respectively, whereas inflatin E (**5**) is a heterodimer originating from **3** and **9**, all via an ether linkage between the two moieties. To our knowledge, inflatins D−F (**4**−**6**) are the first dimeric isochlamydosporols identified from natural sources.

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. ^{1}H 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; $CDCl_3$: δ_H 7.26/δ_C 76.7) as references. The HSQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS and HRESIMS data were obtained using an Agilent Accurate-Mass-Q-TOF LC/MS 6520 instrument equipped with an electrospray ionization (ESI) source. The fragmentor and capillary voltages were kept at 125 and 3500 V, respectively. Nitrogen was supplied as the nebulizing and drying gas. The temperature of the drying gas was set at 300 °C. The flow rate of the drying gas and the pressure of the nebulizer were 10 L/min and 10 psi, respectively. All MS experiments were performed in positive ion mode. Full-scan spectra were acquired over a scan range of m/z 100−1000 at 1.03 spectra/s.

Fungal Material. The culture of *T. inflatum* was isolated from a soil sample on the surface of the fruiting body of *C. sinensis* (Berk.) Sacc. collected in Kangding, Sichuan, People's Republic of China, in May 2005. The isolate was identified by one of the authors (X.L.) on the basis of morphology and sequence (Genbank Accession No. JN003828) analysis of the ITS region of the rDNA and assigned the accession number SCK6-CP14 in X.L.'s culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The fungal strain was cultured on slants of potato dextrose agar at 25 °C for 10 days. Agar plugs were cut into small pieces (about 0.5 × 0.5 × 0.5 cm^3) under aseptic conditions, and 15 pieces were used to inoculate 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. Fermentation was carried out in eight Fernbach flasks (500 mL), 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 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 repeatedly with EtOAc (4 × 1.0 L), and the organic solvent was evaporated to dryness under vacuum to afford the crude extract (6.2 g), which was fractionated by silica gel VLC using petroleum ether−EtOAc gradient elution. The fraction (130 mg) eluted with 30% EtOAc was separated by Sephadex LH-20 column chromatography (CC) eluting with 1:1 CH_2Cl_2−MeOH. The resulting subfractions were combined and further purified by semipreparative RP HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 40% MeOH in H_2O for 5 min, followed by 40−73% over 50 min; 2 mL/min) to afford **4** (15.0 mg, t_R 48.90 min), **5** (8.0 mg, t_R 46.71 min), and **6** (12.5 mg, t_R 49.70 min). The fraction (120 mg) eluted with 35% EtOAc was separated by Sephadex LH-20 CC eluting with 1:1 CH_2Cl_2−MeOH. The resulting subfractions were combined and purified by RP HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 70% MeOH in H_2O for 5 min, followed by 70−95% over 35 min; 2 mL/min) to afford **1** (22.0 mg, t_R 28.32 min) and **2** (4.0 mg, t_R 32.31 min). The fraction (140 mg) eluted with 45% EtOAc was separated by Sephadex LH-20 CC eluting with MeOH, and the resulting subfractions were purified by RP HPLC (20% MeOH in H_2O for 2 min, followed by 20−62% over 38 min; 2 mL/min) to afford a mixture of **3** and **9** (22.5 mg, t_R 36.14 min), which was separated again by RP HPLC (Kromasil 100-5 C_{18} column; 5 μm; 4.6 × 250 mm; 10% MeOH, 10.4% ACN, and 0.5% THF in H_2O for 35 min; 1 mL/min) to afford **3** (7.5 mg, t_R 34.11 min) and **9** (8.5 mg, t_R 34.26 min). The fraction (110 mg) eluted with 50% EtOAc was purified by RP HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 68% MeOH in H_2O for 2 min, followed by 68−84% over 30 min; 2 mL/min) to afford **7** (15.1 mg, t_R 22.37 min) and **8** (3.1 mg, t_R 27.26 min).

*Inflatin A (**1**):* pale yellow oil; $[\alpha]^{23}_D$ +3.2 (*c* 1.6, MeOH); UV (MeOH) λ_{max} (log ε) 223 (3.24), 268 (3.18), 280 (3.26) nm; CD (*c* 4.5 × 10^{-3} M, MeOH) λ_{max} ($\Delta\varepsilon$) 209 (−2.72), 223 (+1.17), 268 (+0.12); IR (neat) ν_{max} 3384 (br), 2936, 2862, 1616, 1520, 1453, 1392, 1355, 1234, 1120, 1096 cm^{-1}; ^{1}H and ^{13}C NMR and HMBC data see Table 1; NOESY correlations (acetone-d_6, 600 MHz) H-3 ↔ H-18b, H_3-19, H-1′; H-18b ↔ H-3, H_3-19, H-1′; H_3-19 ↔ H-3, H-18b, H_3-20; H_3-20 ↔ H_3-19; H-1′ ↔ H-3, H-18b; HRESIMS m/z 443.2788 (calcd for $C_{27}H_{39}O_5$, 443.2792).

Absolute Configuration of the Tertiary Alcohol in 1 (refs 11, 12). A sample of **1** (0.5 mg) was dissolved in a dry solution of $[Rh_2(OCOCF_3)_4]$ complex (1.5 mg) in CH_2Cl_2 (200 μL). The first CD

spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary (ca. 10 min after mixing). The inherent CD was subtracted. The observed sign of the E band at ca. 350 nm in the induced CD spectrum was correlated to the absolute configuration of the C-16 tertiary alcohol moiety.

Inflatin B (**2**): pale yellow oil; $[\alpha]^{23}_{D}$ −1.5 (*c* 0.7, MeOH); UV (MeOH) λ_{max} (log ε) 227 (3.45), 281 (3.20) nm; IR (neat) ν_{max} 3365 (br), 2932, 2862, 1721, 1687, 1603, 1519, 1454, 1390, 1238, 1120, 1096 cm^{-1}; ^1H and ^{13}C NMR and HMBC data see Table 1; NOESY correlations (acetone-d_6, 500 MHz) H-3 ↔ H-18b, H_3-19, H-1′; H-18b ↔ H-3, H_3-19, H-1′; H_3-19 ↔ H-3, H-18b, H_3-20; H_3-20 ↔ H_3-19; H-1′ ↔ H-3, H-18b; HRESIMS *m*/*z* 485.2890 (calcd for $C_{29}H_{41}O_6$, 485.2897).

Inflatin C (**3**): white powder; $[\alpha]^{23}_{D}$ −37.0 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 213 (3.84), 282 (3.80) nm; CD (*c* 1.2 × 10^{-3} M, MeOH) λ_{max} ($\Delta\varepsilon$) 215 (−0.78), 231 (+0.56), 272 (+0.30); IR (neat) ν_{max} 3349 (br), 2979, 1703, 1654, 1566, 1461, 1413, 1263, 1033 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) δ 5.72 (1H, d, *J* = 4.0 Hz, H-5), 5.60 (1H, d, *J* = 4.0 Hz, OH-5), 5.45 (1H, s, H-2), 4.53 (1H, qd, *J* = 5.5, 2.0 Hz, H-6), 3.88 (3H, s, H_3-11), 2.47 (1H, qd, *J* = 6.0, 2.0 Hz, H-7), 1.19 (3H, d, *J* = 5.5, H_3-9), 1.09 (3H, d, *J* = 6.0, H_3-10); ^{13}C NMR (acetone-d_6, 100 MHz) δ 169.7 (C, C-3), 164.5 (C, C-8), 163.4 (C, C-1), 109.4 (C, C-4), 88.4 (CH, C-2), 87.4 (CH, C-5), 64.7 (CH, C-6), 56.7 (CH_3, C-11), 36.2 (CH, C-7), 17.4 (CH_3, C-9), 11.4 (CH_3, C-10); NOED data (acetone-d_6, 500 MHz) H-5 ↔ H_3-10; HRESIMS *m*/*z* 227.0911 (calcd for $C_{11}H_{15}O_5$, 227.0914).

Absolute Configuration of the Secondary Alcohol in 3 (refs 11, 12). A sample of **3** (0.5 mg) was dissolved in a dry solution of $[Rh_2(OCOCF_3)_4]$ complex (1.5 mg) in CH_2Cl_2 (200 μL). 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 E band at ca. 350 nm in the induced CD spectrum was correlated to the absolute configuration of the C-5 secondary alcohol moiety.

Inflatin D (**4**): white needles (acetone−H_2O); mp 287−289 °C; $[\alpha]^{23}_{D}$ −2.0 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.19), 223 (4.25), 278 (4.39) nm; IR (neat) ν_{max} 2984, 1724, 1656, 1568, 1460, 1391, 1265, 1083 cm^{-1}; ^1H and ^{13}C NMR and HMBC data see Table 2; HRESIMS *m*/*z* 435.1647 (calcd for $C_{22}H_{27}O_9$, 435.1650).

X-ray Crystallographic Analysis of 4 (ref 26). Upon crystallization from acetone−H_2O (10:1) using the vapor diffusion method, colorless crystals were obtained for **4**. A crystal (0.77 × 0.59 × 0.51 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 Cu Kα radiation, λ = 1.54178 Å, at 173(2) K. Crystal data: $C_{22}H_{26}O_9$, *M* = 434.43, space group orthorhombic, *P*2(1)2(1)2; unit cell dimensions *a* = 11.679(2) Å, *b* = 19.690(4) Å, *c* = 4.5053(9) Å, *V* = 1036.0(4) $Å^3$, *Z* = 2, D_{calcd} = 1.393 mg/m^3, μ = 0.913 mm^{-1}, *F*(000) = 460. The structure was solved by direct methods using SHELXL-97[27] 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 using the Siemens Area Detector Absorption Program (SADABS).[28] The 7197 measurements yielded 1873 independent reflections after equivalent data were averaged, and Lorentz and polarization corrections were applied. The final refinement gave R_1 = 0.0293 and wR_2 = 0.0700 [$I > 2\sigma(I)$].

Inflatin E (**5**): white needles (acetone−H_2O); mp 274−276 °C; $[\alpha]^{23}_{D}$ +24.0 (*c* 0.9, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.25), 223 (4.10), 278 (4.26) nm; IR (neat) ν_{max} 2979, 1728, 1657, 1569, 1460, 1390, 1261, 1081 cm^{-1}; ^1H and ^{13}C NMR and HMBC data see Table 2; HRESIMS *m*/*z* 435.1650 (calcd for $C_{22}H_{27}O_9$, 435.1650).

X-ray Crystallographic Analysis of 5 (ref 29). Upon crystallization from acetone−H_2O (10:1) using the vapor diffusion method, colorless crystals were obtained for **5**. A crystal (0.72 × 0.12 × 0.09 mm) was separated from the sample and mounted on a glass fiber, and data were collected using a Rigaku RAPID IP diffractometer with graphite-monochromated Mo Kα radiation, λ = 0.71073 Å at 173(2) K. Crystal data: $C_{25}H_{32}O_{10}$, *M* = 492.51, space group orthorhombic, *P*2(1)2(1)2(1); unit cell dimensions *a* = 11.561(2) Å, *b* = 11.932(2) Å, *c* = 18.592(4) Å, *V* = 2564.5(9) $Å^3$, *Z* = 4, D_{calcd} = 1.276 mg/m^3, μ = 0.099 mm^{-1}, *F*(000) = 1048. The structure was solved by direct methods using SHELXL-97[27] 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 using the Siemens Area Detector Absorption Program (SADABS).[28] The 8609 measurements yielded 2615 independent reflections after equivalent data were averaged, and Lorentz and polarization corrections were applied. The final refinement gave R_1 = 0.0946 and wR_2 = 0.2184 [$I > 2\sigma(I)$].

Inflatin F (**6**): white powder; $[\alpha]^{23}_{D}$ +26.0 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.24), 227 (4.23), 285 (4.38) nm; IR (neat) ν_{max} 2974, 1725, 1654, 1576, 1458, 1389, 1247, 1082 cm^{-1}; ^1H and ^{13}C NMR and HMBC data see Table 2; NOESY correlations (acetone-d_6, 500 MHz) H-5/5′ ↔ H_3-9/9′; H-6/6′ ↔ H_3-10/10′; H-7/7′ ↔ H_3-9/9′; H_3-9/9′ ↔ H-5/5′, H-7/7′; H_3-10/10′ ↔ H-6/6′; HRESIMS *m*/*z* 435.1653 (calcd for $C_{22}H_{27}O_9$, 435.1650).

Aphidicolin (**7**): ^1H and ^{13}C NMR and MS data were consistent with literature values.[8]

Aphidicolin-17-monoacetate (**8**): ^1H and ^{13}C NMR and MS data were consistent with literature values.[9]

Isochlamydosporol (**9**): ^1H and ^{13}C NMR and MS data were consistent with literature values.[10]

MTS Assay (refs 30, 31). 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 100 μL of medium containing 0.1% DMSO, or appropriate concentrations of the test compounds and the positive control paclitaxel (Sigma) (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 was 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.

■ ASSOCIATED CONTENT

Supporting Information. ^1H and ^{13}C NMR spectra of **1**−**6** and CD spectra of **1**−**6**, **9**, and Rh complexes of **3** and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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