DOI: 10.1002/ejoc.200801085

Spicochalasin A and New Aspochalasins from the Marine-Derived Fungus *Spicaria elegans*

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Keywords: Spicochalasin A / Cytochalasans / Antibiotics / Natural products / Marine fungi / Antitumor agents

Different culture conditions directed by the OSMAC (one strain-many compounds) approach drastically modified the metabolites of the fungus *Spicaria elegans*, which yielded the novel spicochalasin A (**1**), five new aspochalasins M–Q (**2**–**6**), and two known aspochalasins (**7** and **8**). The gross structures of **1**–**6** were elucidated by 1D and 2D NMR and MS methods, and their absolute configurations were determined by X-ray diffraction and the Mosher ester method. Spicochalasin A (**1**) has a unique pentacyclic system and was found to be moderately cytotoxic towards human leukemic HL-60 cells with an IC_{50} value of 19.9 μM.

Introduction

The cytochalasans are a group of secondary fungal metabolites well known for their distinctive biological activities.[1] Several members of this family have been identified as antibacterial, antiviral, antiinflammatory, and antitumor agents.[2] The common structural feature of the group is an isoindolone moiety bearing a benzyl group (cytochalasins), a 2-methylpropyl group (aspochalasins), an (indol-3-yl)-methyl group (chaetoglobosins),[2] or a methyl group (alachalasins)[3] at the C-3 position and a macrocyclic ring connecting the C-8 and C-9 positions. Isotope labeling experiments have revealed that the biosynthesis of cytochalasans involves the formation of an acetyl- and methionine-derived polyketide chain and the attachment of an amino acid such as phenylalanine, leucine, or tryptophan.[2]

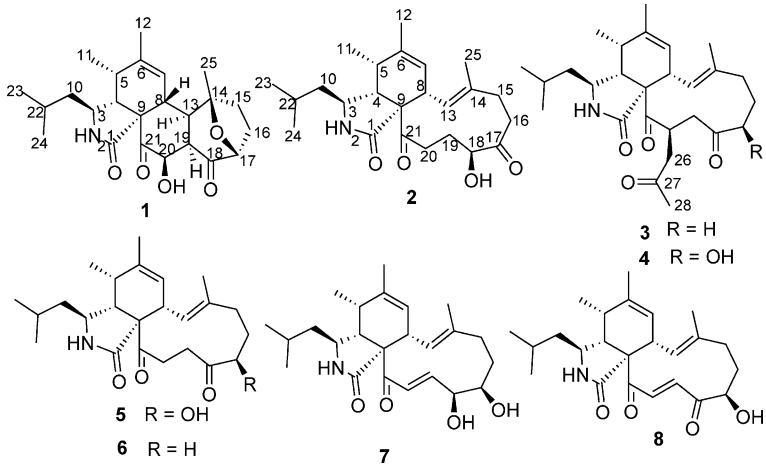

Figure 1. Structures of **1**–**8**.

In our ongoing search for new bioactive secondary metabolites from marine fungi, nine new cytochalasins, Z_7–Z_{15}, have been isolated from the marine-derived fungus *Spicaria elegans*,[4,5] and some of these compounds exhibited interesting cytotoxicities against the A-549 cell line. It is believed that many fungi have a wealth of secondary metabolites depending on the growth conditions present in the

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.200801085.

habitat. The discovery of natural products under laboratory conditions is clearly dependent on the effective simulation of the native growth conditions by the OSMAC (one strain-many compounds) approach.[6] In order to find more diverse cytochalasins from this strain, we employed the OSMAC approach by varying the culture conditions of *S. elegans*. This approach induced a shift in the biosynthesis of cytochalasans. The dominant production of 10-phenylcytochalasins[4,5] was abolished under the new culture conditions, and leucine was dramatically involved in a new biosynthetic pathway leading to the production of the novel spicochalasin A (**1**) and aspochalasins (**2**–**6**) (Figure 1). We report herein the culture of *S. elegans* by the OSMAC approach and the isolation and structural elucidation of the five new compounds including their absolute configuration determination. The inhibitory activities of these compounds against the growth of four cancer cell lines were also evaluated.

Results and Discussion

A previous study has shown that the fungal strain *S. elegans* produced 10-phenylcytochalasins (Z_7–Z_{15}).[4,5] We investigated the metabolites of this strain when cultured in different media by the OSMAC approach. We screened a panel of extracts prepared under 10 different culture conditions (five different media, stationary or shake culture) by LC-MS, with particular attention being given to nitrogen-containing metabolites. In a new medium (composed of 2% soluble starch, 1.5% soybean flour, 0.5% yeast ext., 0.2% peptone, and sea water), we incubated the cultures at 28 °C for 8 d on a rotary shaker (128 rpm).

We then undertook an analysis of the HPLC profiles of the extract of *S. elegans*. These results (see Scheme S1) clearly depicted a dramatic shift in the profiles of the secondary metabolites obtained compared to those obtained under our initial conditions. The initial cultures appeared to be rich in cytochalasins, which mainly eluted before 45 min by HPLC. Under the new culture conditions, new, intense, HPLC peaks appeared mainly after 45 min, which were identified as previously unknown nitrogen-containing compounds by LC-MS analysis. In order to obtain milligram quantities of the new metabolites and characterize them, we prepared scale-up cultures (60 L). We purified the EtOAc extract of the combined broth and mycelia, shown by HPLC to contain the interesting compounds, by normal and reversed-phase chromatography. We obtained spicochalasin A (**1**) and four new aspochalasins (**2**–**6**) along with two known ones (**7** and **8**).

Structure Elucidation

We obtained spicochalasin A (**1**) as a colorless solid. HR-ESI-MS gave an exact mass of 416.2434 for $[M + H]^+$ (calcd. 416.2437 for $C_{24}H_{34}NO_5$), suggesting a molecular formula of $C_{24}H_{33}NO_5$. Inspection of the ^1H and ^{13}C NMR

Table 1. ^1H NMR data for **1**–**6**.[a]

Entry	Atom	1	2	3	4	5	6
1	2-NH	6.40, br. s	6.44, br. s	6.94, br. s	7.25, br. s	6.49, br. s	5.96, br. s
2	3	3.21, m	3.17, m	3.04, br. d (10.5)	3.05, br. d (10.5)	3.12, m	3.15, m
3	4	3.12, dd (5.9, 2.8)	2.59, dd (6.4, 2.8)	2.44, m	2.41, dd (4.4, 4.4)	2.45, m	2.62, m
4	5	2.38, m	2.55, m	2.61, m	2.61, m	2.62, m	2.57, m
5	7	5.49, br. s	5.40, br. s	5.37, br. s	5.36, br. s	5.36, br. s	5.39, br. s
6	8	2.37, m	3.05, br. d (10.6)	3.32, br. d (11.0)	3.37, br. d (11.0)	3.26, d (10.6)	3.01, br. d (11.0)
7	10	1.35, m; 1.23, m	1.19, m; 1.12, m	1.84, m; 1.30, m	1.95, m; 1.30, m	1.26, m; 1.18, m	1.19, m; 1.17, m
8	11	1.22, d (7.4)	1.20, d (7.3)	1.17, d (6.9)	1.18, d (6.5)	1.19, d (6.9)	1.19, d (7.3)
9	12	1.79, br. s	1.74, br. s	1.76, br. d	1.76, br. s	1.75, br. s	1.74, br. s
10	13	3.02, m	6.23, d (10.5)	6.07, d (11.0)	6.13, d (11.0)	6.16, d (11.0)	6.22, br. d (10.6)
11	15	1.90, m; 1.65m	2.64, dd (12.3, 9.6); 2.26, m	2.44, m; 2.06, m	2.36, m; 2.17, m	2.40, m; 2.21, m	2.16, ddd (12.4, 3.7, 3.7); 1.99, ddd (12.2, 12.2, 3.7)
12	16	2.47, m; 2.06, m	3.28, dd (11.9, 10.6); 2.20, m	2.26, m; 1.57, m	2.17, m; 1.95, m	2.19, m; 2.03, m	2.26, m; 1.55, m
13	17	4.41, dd (9.6, 2.2)	–	2.10, m; 1.96, m	4.10, m	4.22, br. s	2.63, m; 2.07, dd (16.9, 6.8)
14	18	–	4.15, dd (5.0, 5.0)	–	–	–	–
15	19	3.01, m	2.19, m; 2.00, m	4.39, dd (18.3, 3.7); 2.35, dd (18.8, 5.0)	4.78, dd (19.2, 3.8); 2.24, m	2.84, ddd (12.4, 12.4, 3.6); 2.67, m	2.81, ddd (13.7, 10.9, 2.3); 2.73, ddd (13.7, 8.3, 2.3)
16	20	5.19, br. d (10.1)	3.57, m; 2.21, m	3.29, m	3.25, m	4.37, ddd (18.8, 11.9, 2.7)	3.85, ddd (17.4, 11.5, 2.3); 2.41, ddd (17.4, 8.2, 2.3)
17	22	1.51, m	1.55, m	1.67, m	1.69, m	1.60, m	1.55, m
18	23	0.90, d (6.9)	0.91, d (6.8)	0.97, d (6.4)	0.98, d (6.6)	0.92, d (6.8)	0.90, d (6.8)
19	24	0.92, d (6.4)	0.91, d (6.8)	0.96, d (6.4)	0.98, d (6.6)	0.92, d (6.8)	0.90, d (6.8)
20	25	1.41, s	1.63, br. s	1.37, br. s	1.34, br. s	1.38, br. s	1.36, br. s
21	26	–	–	3.97, dd (18.3, 9.5); 2.23, dd (18.8, 3.7)	4.12, m; 2.26, m	–	–
22	28	–	–	2.14, s	2.15, s	–	–

[a] Signals are given as δ_H, multiplicity (J in Hz).

spectra (Tables 1 and 2) in combination with DEPT and ^{1}H-^{13}C HMQC spectra revealed the presence of five methyl groups [11-CH_3: δ_H = 1.22 (d) ppm, δ_C = 13.1 ppm; 12-CH_3: δ_H = 1.79 (s) ppm, δ_C = 20.0 ppm; 23-CH_3: δ_H = 0.90 (d) ppm, δ_C = 23.4 ppm; 24-CH_3: δ_H = 0.92 (d) ppm, δ_C = 21.3 ppm; 25-CH_3: δ_H = 1.41 (s) ppm, δ_C = 21.3 ppm], three multiplet methylene groups (10-CH_2: δ_C = 47.4 ppm; 15-CH_2: δ_C = 41.0 ppm; 16-CH_2: δ_C = 29.8 ppm), a double bond [6-C: δ_C = 140.3 (qC) ppm; 7-CH: δ_H = 5.49 (br. s) ppm, δ_C = 125.3 ppm], two oxygen-substituted methine groups [17-CH: δ_H = 4.41 (dd) ppm, δ_C = 79.8 ppm; 20-CH: δ_H = 5.19 (br. d) ppm, δ_C = 71.4 ppm], an oxygen-substituted quaternary carbon atom (14-C: δ_C = 82.5 ppm), an amide carbonyl group (NH-2: δ_H = 6.44 ppm; C-1: δ_C = 172.9 ppm), and two ketone carbonyl groups (C-18: δ_C = 213.8 ppm; C-21: δ_C = 204.1 ppm). Moreover, we detected resonance signals for an sp^3-hybridized quaternary carbon atom and seven sp^3-hybridized methine groups. Since 4 out of 9 unsaturations were accounted for, it was inferred that **1** had five rings. ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMBC NMR experiments allowed for the deduction of the gross structure of **1** as shown in Figure 2. The spin system beginning with 23-, 24-CH_3 and continuing through to 11-CH_3, as well as the fragments from 7-CH to 20-CH and from 15-CH_2 to 17-CH, could be concluded on the basis of the ^{1}H-^{1}H COSY correlations (Figure 2). The key HMBC correlations from H-12 to C-5, C-7, and C-6, from H-7, H-5, and NH-2 to C-9, from H-3 to C-1, and from NH-2 to C-4 established the perhydroisoindol-1-one skeleton. The diagnostic cross peaks from H-13 to C-9 and from H-19 to C-21 in the HMBC spectrum, along with the fragment from 7-CH to 20-CH, deduced from ^{1}H-^{1}H COSY correlations, indicated a cyclohexanone substructure fused with the perhydroisoindol-1-one at C-8 and C-9. Further analysis of the HMBC correlations from H-25 to C-13, C-15, and C-14, and from H-16 and H-13 to C-18 indicated a cycloheptanone substructure fused with the above-mentioned cyclohexanone substructure at C-13 and C-19, and the remaining signal from H-17 to C-14 identified an oxo bridge between the two oxygenated carbon atoms, C-14 and C-17. And finally, to satisfy the element of unsaturation, the remaining oxygenated methine group (20-CH) must be connected with a hydroxy group. The perhydroisoindol-1-one substructure fused with the cyclohexanone and cycloheptanone substructures, along with the oxo bridge between C-14 and C-17, made up a polycyclic skeletal structure as shown in Figure 2. Even though aspergillin PZ[7] has been reported, the oxo bridge between C-14 and C-17, resulting in the pentacyclic system of **1**, is a unique feature to the best of our knowledge.

Table 2. ^{13}C NMR data for **1**–**6**.

Entry	Atom	**1**	**2**	**3**	**4**	**5**	**6**
1	1	172.9, s	175.3, s	176.4, s	176.7, s	176.3, s	175.7, s
2	3	51.5, d	50.8, d	51.6, d	52.0, d	51.0, d	50.6, d
3	4	46.2, d	52.1, d	55.3, d	55.3, d	55.2, d	52.6, d
4	5	34.2, d	35.2, d	35.5, d	35.7, d	35.4, d	35.2, d
5	6	140.3, s	140.0, s	139.5, s	139.8, s	139.4, s	139.6, s
6	7	125.3, d	125.6, d	126.0, d	125.6, d	126.0, d	125.9, d
7	8	45.1, d	43.5, d	42.4, d	42.5, d	42.1, d	43.2, d
8	9	65.8, s	66.9, s	68.2, s	68.9, s	68.6, s	66.9, s
9	10	47.4, t	48.5, t	47.4, t	47.2, t	48.8, t	48.6, t
10	11	13.1, q	13.5, q	13.5, q	13.6, q	13.4, q	13.4, q
11	12	20.0, q	19.8, q	20.2, q	20.3, q	19.9, q	19.8, q
12	13	37.6, d	123.8, d	125.1, d	125.1, d	124.9, d	124.3, d
13	14	82.5, s	137.1, s	137.9, s	136.7, s	136.8, s	137.7, s
14	15	41.0, t	36.8, t	41.8, t	37.0, t	37.1, t	41.2, t
15	16	29.8, t	26.4, t	23.1, t	29.9, t	30.5, t	20.0, t
16	17	79.8, d	216.0, s	41.6, t	74.5, d	74.4, d	39.1, t
17	18	213.8, s	77.4, d	209.5, s	209.8, s	209.6, s	208.3, s
18	19	53.0, d	34.5, t	42.4, t	33.3, t	32.7, t	37.9, t
19	20	71.4, d	37.5, t	43.1, d	42.1, d	33.7, t	37.6, t
20	21	204.1, s	214.5, s	208.3, s	209.2, s	209.1, s	208.1, s
21	22	24.9, d	25.0, d	25.3, d	24.1, d	25.1, d	25.1, d
22	23	23.4, q	23.5, q	24.0, q	25.3, q	23.7, q	23.5, q
23	24	21.3, q	21.5, q	21.1, q	21.1, q	21.4, q	21.5, q
24	25	21.3, q	16.2, q	14.8, q	18.7, q	18.5, q	14.7, q
25	26	–	–	44.3, t	44.3, t	–	–
26	27	–	–	207.1, s	207.2, s	–	–
27	28	–	–	29.8, q	29.7, q	–	–

NOESY correlations (Figure 2) among H-10, H-5, H-4 and H-8 and between H-3 and H-11 demonstrated that the relative configurations of the perhydroisindol-1-one moiety were in accord with those of reported cytochalasans[4,5] and the signal between H-25 and H-8 indicated that H-25 pointed toward H-8. The other NOESY correlations between H-20 and H-13, between H-13 and H-15, and between H-15 and H-19 indicated that the fused and bridged rings were oriented in a *trans* fashion about the central tetrahydropyrane ring (Figure 2). We established the absolute configuration of **1** by a convenient Mosher ester

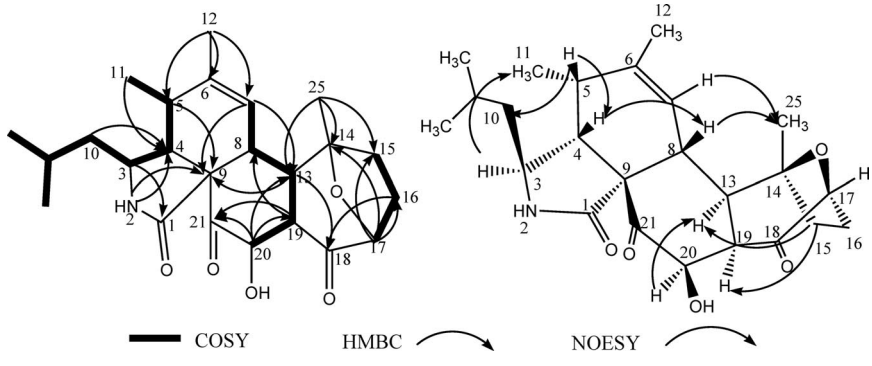

Figure 2. ^{1}H-^{1}H COSY correlations and key HMBC correlations of **1**.

method using the (*S*)- and (*R*)-MTPA esters. We prepared Mosher ester derivatives (**1a** and **1b**) of **1**. The positive and negative value disposition of $\Delta\delta^{S-R}$ (Figure 3) in the molecule established the (20*R*) absolute configuration. Therefore, the complete absolute stereochemistry of **1** could be assigned as (3*S*,4*R*,5*S*,8*S*,9*S*,13*R*,14*S*,17*R*,19*R*,20*R*).

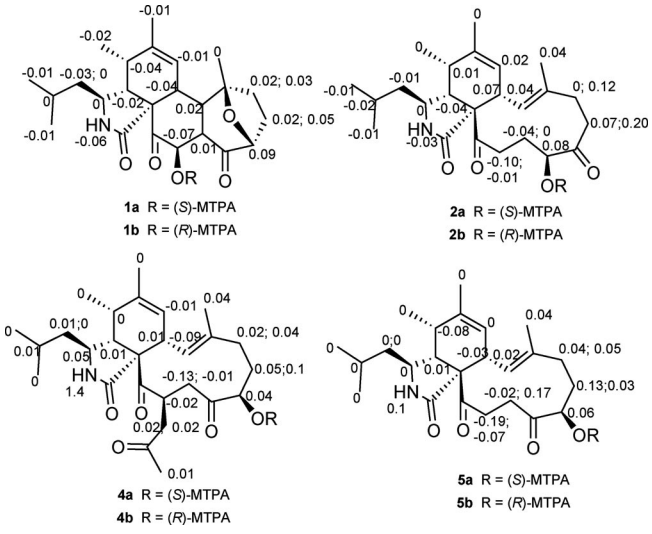

Figure 3. $\Delta\delta$ values [$\Delta\delta^{S-R}$ (in ppm) = $\delta^S - \delta^R$] obtained for the (*S*)- and (*R*)-MTPA esters of **1**, **2**, **4** and **5**.

We obtained aspochalasin M (**2**) as a colorless solid. HR-ESI-MS gave an exact mass of 402.2656 for $[M + H]^+$ (calcd. 402.2644 for $C_{24}H_{36}NO_4$), suggesting a molecular formula of $C_{24}H_{35}NO_4$ for **2**. The ^1H NMR spectrum recorded in $CDCl_3$ intuitively revealed five methyl groups (Table 1), two olefinic protons [H-13: δ_H = 6.23 (d) ppm; H-7: δ_H = 5.40 (br. s) ppm], an amide group (NH: δ_H = 6.44 ppm), five methine protons, along with another 12 protons (Table 1). These features characteristically revealed the structure of **2** as possessing a (2-methylpropyl)isoindolone moiety, consistent with an aspochalasin skeleton.[8] We also confirmed this with the ^{13}C NMR and DEPT spectra (Table 2). Another 10 resonance signals in the ^{13}C NMR spectrum revealed that **2** possessed an 11-membered macrocycle containing a methyl group (C-25: δ_C = 16.2 ppm), a double bond [C-13: δ_C = 123.8 (CH) ppm; C-14: δ_C = 137.1 (qC) ppm] and two carbonyl groups (C-17: δ_C = 216.0 ppm; C-21: δ_C = 214.5 ppm). The planar structure of **2** was elucidated by the interpretation of extensive ^1H-^1H COSY and HMBC experiments (see the Supporting Information). ^1H-^1H COSY cross peaks for the H-20/H-19, H-19/H-18, and H-15/H-16 pairs established the position of the hydroxy group at C-18 and located the keton carbonyl group at C-17. We further confirmed this with the X-ray diffraction structure of **2** (Figure 4). Its X-ray diffraction also demonstrated the relative configurations. We established the absolute configuration of **2** by a convenient Mosher ester method using the (*S*)- and (*R*)-MTPA esters. We prepared Mosher ester derivatives (**2a** and **2b**) of **2**. The positive and negative value disposition of $\Delta\delta^{S-R}$ in the molecule established the (18*S*) configuration (Figure 3). Therefore, the complete absolute configurations of **1** could be assigned as (3*S*,4*R*,5*S*,8*S*,9*S*,13*E*,18*S*).

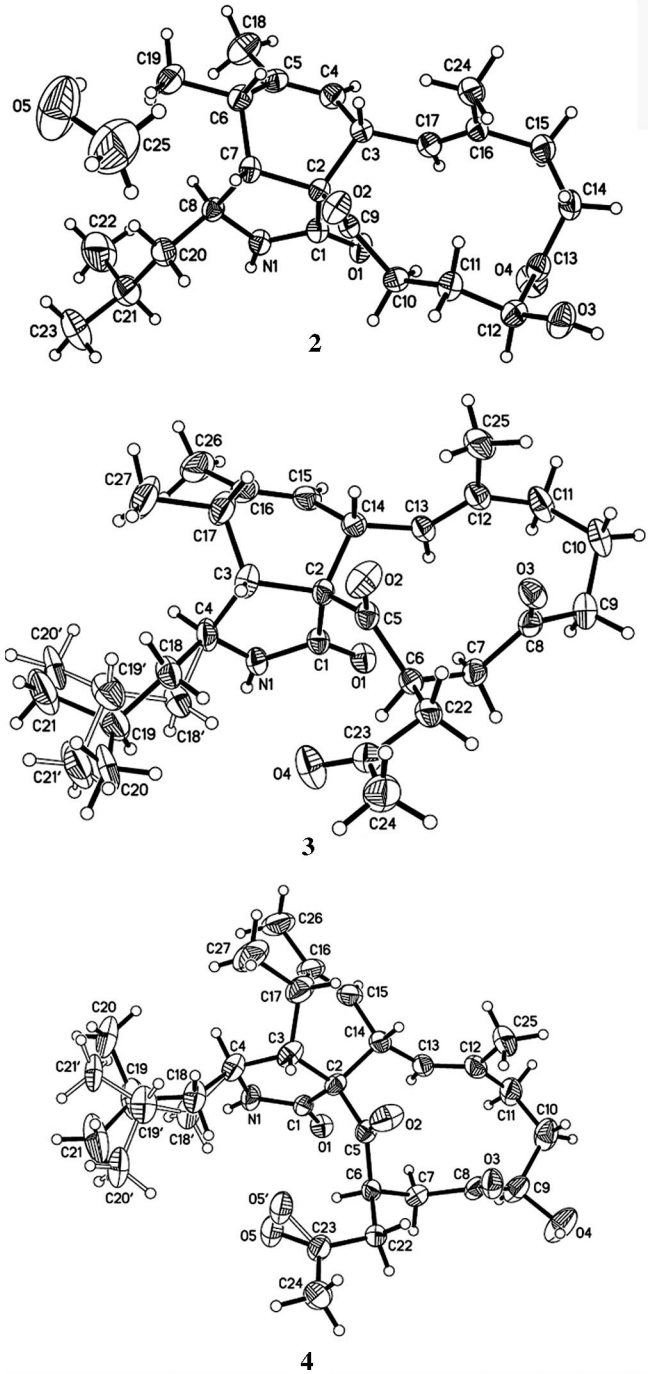

Figure 4. Final X-ray structures of **2**–**4**.

The NMR data of aspochalasins M–Q (**3**–**6**) was in full agreement with the previously reported values for aspochalasins.[8] In contrast to **2**, both **3** and **4** contained one more acetonyl group [28-CH_3: δ_H = 2.14 (s) ppm, δ_C = 29.8 ppm; C-27: δ_C = 207.1 ppm; 26-CH_2: δ_C = 207.1 ppm, δ_H = 3.97 (dd), 2.23 (dd) ppm, δ_C = 44.3 ppm in **3**; 28-CH_3: δ_H = 2.15 (s) ppm, δ_C = 29.7 ppm; C-27: δ_C = 207.2 ppm; 26-CH_2: δ_H = 4.12 (m) 2.26 (m) ppm, δ_C = 44.3 ppm in **4**]. We con-

firmed that the acetonyl group was substituted at C-20 in **3** and **4** with HMBC correlations between H-20 and C-9 and between H-26 and C-21 and with ^{1}H-^{1}H COSY correlations for the H-19/H-20 and H-15/H-16/H-17 combinations. Moreover, **4** had a hydroxy group at the C-17 position. We also confirmed the structures of **3** and **4** with X-ray diffraction (Figure 4). Compound **5** is an isomer of **2** as they share the same molecular formula, $C_{24}H_{35}NO_4$. ^{1}H-^{1}H COSY correlations for the H-20/H-19 and H-17/H-16/H-15 combinations revealed the carbonyl group at C-18 and the hydroxy group at C-17. Compound **6** had a molecular formula of $C_{24}H_{35}NO_3$, which had one oxygen atom less than **5**. Compared to those of **5**, the ^{1}H and ^{13}C NMR spectra of **6** showed the absence of an oxygenated methine group, and ^{1}H-^{1}H COSY correlations for the H-20/H-19 and H-17/H-16/H-15 combinations revealed that one of the carbonyl groups was still at C-18 (Table 2). The X-ray diffraction structure demonstrated that the relative configurations of **3** and **4** were in accord with those of **2**. It was reported that the absolute and relative configuration of the isoindolone remained the same[4,5] because of the diastereofacial selectivity of the cycloaddition during the biosynthesis of cytochalasans. Compounds **2**–**6** should have the same absolute configuration about the isoindolone moiety. Therefore, (20*R*) and (20*R*,17*R*) absolute configurations could be deduced for **3** and **4**, respectively. We prepared Mosher ester derivatives (**4a** and **4b**) of **4**, and the positive and negative value disposition of $\Delta\delta^{S-R}$ (Figure 3) was in full agreement with the (17*R*) configuration. In order to demonstrate the absolute configuration of C-17 in **5**, we prepared Mosher ester derivatives (**5a** and **5b**) of **5**. A detailed analysis of the positive and negative value disposition of $\Delta\delta^{S-R}$ (Figure 3) showed a result similar to that of **4a** and **4b**. Thereofore, we concluded that **5** also had a (17*R*) absolute configuration. The oxo bridge of **1** between C-14 and C-17 in the cycloheptanone moiety was found for the first time. The ten contiguous stereocenters in structure of **1** might be derived from aspochalasin B (**8**) by an intramolecular cyclization following an oxidation reaction. A proposed biogenetic pathway of **1** is shown in Figure 5. Upon the treatment of mixture **8** with acetone, MeOH and H_2O at room temperature, we obtained **4** (see the Supporting Information), which must be formed in a Michael addition of **8**. This experiment gave evidence for **3** and **4** being artifacts, as the acetonyl group was added during the extraction procedure, which included crushing the mycelium in acetone.

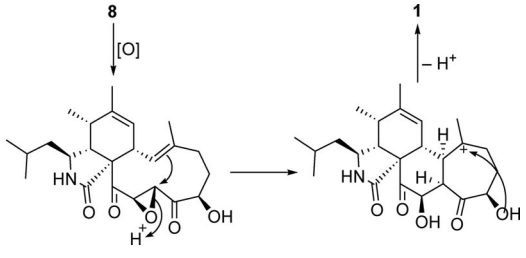

Figure 5. Proposed biogenetic pathway of spicochalasin A (**1**).

Two other known compounds were identified as aspochalasins D (**7**)[9] and B (**8**)[10] by a comparison of their spectroscopic data with the literature values.

Biological Activity

All compounds were evaluated for their growth inhibition against the A-549, BEL-7402, P388 and HL-60 cell lines by the MTT method (Table 3). Spicochalasin A (**1**) and aspochalasin M (**2**) showed modest activity against HL-60 cells, with IC_{50} values of 19.9 and 20.0 μM, respectively. Compounds **7** and **8** inhibited the growth of the four cell lines. Despite the structural differences in their macrocycles, these results strongly implied that the α,β-unsaturated ketone may be an essential part of the pharmacophore.

Table 3. Growth inhibition of **1**–**8** against four cancer cell lines.

Entry	Compounds	Cytotoxicity (IC_{50} in μM) MOLT4 cells	A-549 cells	HL-60 cells	BEL-7402 cells
1	**1**	>100	>100	19.9	>100
2	**2**	>100	>100	20.0	>100
3	**3**–**6**	>100	>100	>100	>100
4	**7**	14.5	6.4	16.3	2.3
5	**8**	13.0	19.6	11.6	>100

Conclusions

Cytochalasans are a large family, with more than 100 members having been isolated. It is reported that only 21 (asopochalasins A–L,[11–16] phomacins A–C[17] and aspochalamin A–D[18]) of them have a leucine residue attached. Compounds **1**–**6** are six new members of this family. Among them, spicochalasin A (**1**) with a pentacyclic system, belongs to an unusual type of aspochalasins, of which only two examples have been reported prior to this new compound (**1**). The absolute configuration of the perhydroisoindol-1-one moiety of aspochalasins has been presumed to be the same as that of 10-phenylcytochalasins because of their similar biosynthetic pathway by a hypothetical Diels–Alder-type reaction.[19] In this paper, the absolute configuration of aspochalasins (such as **2**) was determined for the first time, and this finding verified the biosynthetic pathway of the cytochalasans.

To obtain a variety of cytochalasans, P450 enzyme inhibitors and F-action inhibitors have been used to challenge the cultures of *Phoma* sp. SNF-1778[20] and *Phomospis asparagi*,[21] respectively. Changes in culturing parameters has been used to trigger the expression of a silent gene discovered by genome mining.[22,23] We dramatically modified the biosynthetic pathway of cytochalasans in the strain *S. elegans* by manipulating the culture conditions and verified the importance of the OSMAC approach. Different culture conditions directed by the OSMAC approach can selectively regulate the use of amino acids involved in the biosynthesis of cytochanlasans. To the best of our knowledge,

this is the first report that a fungus can produce two types of cytochalasans, one bearing a phenylalanine residue and the other bearing a leucine residue. The two biosynthetic pathways for 10-phenylcytochalasins and aspochalasins may coexist in the gene clusters of *S. elegans*. This approach may broaden the diversity of active cytochalsans.

Experimental Section

General: Optical rotations were obtained with a Jasco P-1020 digital polarimeter. UV spectra were recorded with a Beckman DU® 640 spectrophotometer. IR spectra were taken with a Nicolet Nexus 470 spectrophotometer in KBr discs. ^{1}H, ^{13}C NMR, DEPT and 2D NMR spectra were recorded with a Jeol JNM-ECP 600 spectrometer by using TMS as an internal standard, and chemical shifts were recorded as δ values; all spectra were recorded at T = 25 °C. NOESY experiments were carried out by using a mixing time of 0.5 s. ESI-MS was measured with a Q-TOF Ultima Global GAA076 LC mass spectrometer. Semiprepartive HPLC was performed by using an ODS column [Shin-pak ODS (H), 10 × 250 mm, 5 μM, 4 mL/min]. Analytical HPLC was performed by using an ODS column [Shin-pak ODS (H), 0.5 mm×250 mm, 5 μM, H_2O/CH_3OH, 1 mL/min].

X-ray Structure Determination: X-ray diffraction intensity data were collected with a MAC DIP-2030K diffractometer using graphite-monochromater Mo-K_α radiation (λ = 0.71073 Å) by the ω-scan technique [scan width 0–180°, $2\theta \leq 50°$]. Hydrogen positions were found from difference Fourier maps and geometric calculations. All calculations were carried out with a personal computer using the SHELX-97 program system. Aspochalasin M (**2**): Altogether 2400 reflections were collected, of which 1556 with $|F|^2 \geq 2\sigma|F|^2$ were observed. The structure was solved by direct methods and refined by the block-matrix least-squares procedure to R_1 = 0.0479, wR_2 = 0.1298. Aspochalasin N (**3**): Altogether 2296 reflections were collected, of which 1787 with $|F|^2 \geq 2\sigma|F|^2$ were observed. The structure was solved by direct methods and refined by the block-matrix least-squares procedure to R_1 = 0.0454, wR_2 = 0.1313. Aspochalasin O (**4**): Altogether 2417 reflections were collected, of which 1799 with $|F|^2 \geq 2\sigma|F|^2$ were observed. The structure was solved by direct methods and refined by the block-matrix least-squares procedure to R_1 = 0.0620, wR_2 = 0.1907. CCDC-692149 (for **2**), -692150 (for **3**), and -692151 (for **4**) 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.

Fungal Material: The fungus *Spicaria elegans* was isolated from the marine sediments collected in Jiaozhou Bay, China. It was preserved in the China Center for Type Culture Collection (patent depositary number: KLA03 CCTCC M 205049).

Fermentation and Extraction: The fungus was incubated at 28 °C under shaking conditions for 8 d in 400 500 mL conical flasks containing the liquid medium (Type B, 150 mL/flask) composed of 2% soluble starch, 1.5% soybean flour, 0.5% yeast extract, 0.2% peptone and sea water. The fermented whole broth (60 L) was extracted three times with EtOAc to give an EtOAc solution, which was concentrated under reduced pressure to give a crude extract (25.5 g).

Purification: The crude extract (25.5 g) was separated into 5 fractions by silica gel chromatography and eluted with a gradient of $CHCl_3$/MeOH. Subfraction 2, eluted with $CHCl_3$/MeOH (95:5) (2.2 g), was purified into 10 subfractions by silica gel chromatography, eluting with $CHCl_3$/MeOH (100:1). Subfractions 2-9 and 2-10 were further purified by reverse-phase chromatography, eluting with MeOH/H_2O (7:3) to give 12 subfractions. The further purification of subfraction 2-9-9 by extensive HPLC (75% MeOH, 4.0 mL/min) gave **8** (40 mg), **3** (10 mg), and **6** (2.0 mg). The further purification of subfraction 2-10-4 by extensive HPLC (70% MeOH, 4.0 mL/min) gave **4** (20 mg), **5** (10 mg), and **2** (18 mg). Subfraction 2-1 was further purified by extensive HPLC (70% MeOH, 4.0 mL/min) to give **1** (6.7 mg). Subfraction 3, eluted with $CHCl_3$/MeOH (90:10) (0.5 g), was purified into 5 subfractions by silica gel chromatography, eluting with $CHCl_3$/MeOH (50:1). Subfraction 3-3 was further purified by reverse-phase chromatography, eluting with MeOH/H_2O (6:4) to give 13 subfractions. Subfraction 3-3-9 was further purified by extensive HPLC (70% MeOH, 4.0 mL/min) to give **7** (5.6 mg).

Spicochalasin A (1): White powder (MeOH). $[a]_D^{25}$ = –50.0 (c = 0.26, $CHCl_3$). UV (MeOH): λ_{max}(log ε) = 205 (3.12) nm. IR (KBr): $\tilde{\nu}$ = 3440, 2900, 1670, 1665, 1310, 1024 cm^{-1}. ^{1}H and ^{13}C NMR: see Tables 1 and 2. HR-ESI-MS: calcd. for $C_{24}H_{34}NO_5$ [M + H]$^+$ 416.2437; found 416.2434.

Aspochalasin M (2): White powder (MeOH). $[a]_D^{25}$ = –20 (c = 0.7, MeOH). UV (MeOH): λ_{max} (log ε) = 203 (3.06) nm. IR (KBr): $\tilde{\nu}_{max}$ = 3468, 2937, 2958, 2921, 1700, 1695, 1444, 1076 cm^{-1}. ^{1}H and ^{13}C NMR: see Tables 1 and 2. HR-ESI-MS: calcd. for $C_{24}H_{36}NO_4$ [M + H]$^+$ 402.2644; found 402.2656.

Aspochalasin N (3): White powder (MeOH). $[a]_D^{25}$ = –146 (c = 0.05, $CHCl_3$). UV (MeOH): λ_{max}(log ε) = 203 (2.74) nm. IR (KBr): $\tilde{\nu}_{max}$ = 3460, 2934, 2968, 2941, 1721, 1700, 1685, 1424, 1066 cm^{-1}. ^{1}H and ^{13}C NMR: see Tables 1 and 2. HR-ESI-MS: calcd. for $C_{27}H_{40}NO_4$ [M + H]$^+$ 442.2957; found 442.2963.

Aspochalasin O (4): White powder (MeOH). $[a]_D^{25}$ = –82.8 (c = 0.42, $CHCl_3$). UV (MeOH): λ_{max}(log ε) = 204 (2.98) nm. IR (KBr): $\tilde{\nu}_{max}$ = 3462, 2914, 2978, 2947, 1711, 1701, 1675, 1324, 1166 cm^{-1}. ^{1}H and ^{13}C NMR: see Tables 1 and 2. HR-ESI-MS: calcd. for $C_{27}H_{40}NO_5$ [M + H]$^+$ 458.2906; found 458.2916.

Aspochalasin P (5): White powder (MeOH). $[a]_D^{25}$ = –107.7 (c = 0.52, $CHCl_3$). UV (MeOH): λ_{max}(log ε) = 206 (2.77) nm. IR (KBr): $\tilde{\nu}_{max}$ = 3365, 2977, 2921, 2911, 1700, 1685, 1324, 1066 cm^{-1}. ^{1}H and ^{13}C NMR: see Tables 1 and 2. HR-ESI-MS: calcd. for $C_{24}H_{36}NO_4$ [M + H]$^+$ 402.2644; found 402.2651.

Aspochalasin R (6): White powder (MeOH). $[a]_D^{25}$ = –66.8 (c = 0.34, $CHCl_3$). UV(MeOH): λ_{max}(log ε) = 205 (3.52) nm. IR (KBr): $\tilde{\nu}_{max}$ = 3364, 2977, 2922, 2911, 2847, 1701, 1693, 1344, 1066 cm^{-1}. ^{1}H and ^{13}C NMR: see Tables 1 and 2. HR-ESI-MS: calcd. for $C_{24}H_{36}NO_3$ [M + H]$^+$ 386.2695; found 386.2700.

Preparation of the (*S*)- and (*R*)-MTPA Ester Derivatives: Compounds **1** (1.0 mg), **2** (5.0 mg), **4** (1.0 mg), and **5** (1.0 mg) were separately transferred into a clean reaction bottle and dried completely under vacuum. Deuterated pyridine (0.5 mL) and (+)-(*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (1 equiv.) were added to the reaction bottle quickly under an N_2 stream, and the mixture was stirred at room temperature for 24 h. The organic layer was then washed with water, HCl (1 ᵐ), water, $NaHCO_3$ (satd. aq.), and water and then dried (Na_2SO_4) and concentrated under reduced pressure to obtain the ester. Final purification was achieved by HPLC (90% MeOH, 4.0 mL/min) to give the (*R*)-MTPA ester derivatives of **1**, **2**, **4** and **5**, seperatively. The (*S*)-MTPA ester derivatives of **1**, **2**, **4** and **5** were prepared accordingly.

Biological Assays: Cytotoxic activity was evaluated by the MTT method using MOLT4, A-549, HL60 and BEL-7402 cell lines. The

cell lines were grown in RPMI-1640 supplemented with 10% FBS under a humidified atmosphere of 5% CO_2 and 95% air at 37 °C (tsFT210 cell line at 32 °C). An aliquot (200 μL) of those cell suspensions at a density of 5×10^4 cells/mL was plated in 96-well microtiter plates and incubated for 24 h under the above conditions. The test compound solutions (2 μL in DMSO) at different concentrations were then added to each well, and the cells were further incubated for 72 h under the same conditions. An MTT solution (20 μL of 5 mg/mL in RPMI-1640 medium) was added to each well, and the plate was incubated for 4 h. The old medium containing MTT (150 μL) was then gently replaced by DMSO, and the mixture was pipetted to dissolve any formazan crystals which had formed. The absorbance was then determined with a Spectra Max Plus plate reader at 570 nm.

Supporting Information (see footnote on the first page of this article): ^{1}H-^{1}H COSY and HMBC data of **2**–**6**, HPLC analysis of the Michael addition reaction of **8** and ^{1}H NMR data of the (*R*)- and (*S*)-MTPA ester derivatives of **1**, **2**, **4** and **5**.

Acknowledgments

This work was financially supported by the Chinese National Natural Science Fund (No. 30772640), and the Shandong Province (No. Z2006C13). The antitumor assay was performed at the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

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Received: November 5, 2008
Published Online: May 5, 2009

