Aspochalasins I, J, and K: Three New Cytotoxic Cytochalasans of *Aspergillus flavipes* from the Rhizosphere of *Ericameria laricifolia* of the Sonoran Desert[1]

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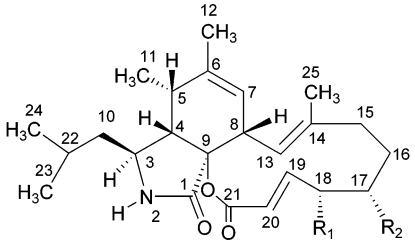
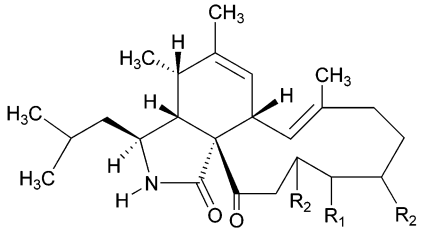
Bioassay-guided fractionation of a cytotoxic EtOAc extract of *Aspergillus flavipes* occurring in the rhizosphere of *Ericameria laricifolia* resulted in the isolation of three new cytochalasans, namely, aspochalasins I (**1**), J (**2**), and K (**3**), and four known cytochalasans, aspochalasins C (**4**), D (**5**), and E (**6**) and TMC-169 (**7**). The structures of compounds **1**−**3** were established on the basis of extensive 1D and 2D NMR spectroscopic analysis. All compounds exhibited weak to moderate cytotoxicity against NCI-H460, MCF-7, and SF-268 cancer cell lines, but none showed significant selectivity.

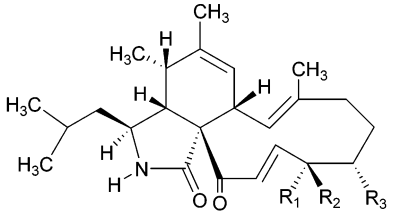
The aspochalasins constitute a subgroup within the small group of fungal secondary metabolites, cytochalasans, many of which are known to exhibit a variety of biological activities.[2] Structurally, all cytochalasans consist of 11-, 13-, or 14-membered carbocyclic (or oxygen-containing) rings connecting the C-8 and C-9 positions of a perhydroisoindol-1-one moiety bearing different substituents at C-3, and in aspochalasins the C-3 substituent is a 2-methylpropyl group. Thus far only 12 aspochalasins are known, and these have been encountered in *Aspergillus microcysticus* (aspochalasins A−D),[3] *Aspergillus* sp. AJ117509 (aspochalasins C, D, and H),[4] an unidentified fungus (aspochalasins C and E),[5] *Aspergillus* sp. (aspochalasins G and F),[6] *A. flavipes* strain TC 1446 (TMC-169),[7] and *Phoma* sp. (phomacins A−C).[8] Aspochalasins are reported to have antibiotic, cytotoxic,[4] and anticytoskeletal[9] activities. In our continuing search for anticancer agents from the rhizosphere microflora of the Sonoran desert plants,[1] an EtOAc extract of *Aspergillus flavipes* (Moniliaceae), a fungus occurring in the rhizosphere of the desert turpentine brush, *Ericameria laricifolia* Nutt. (Asteraceae), exhibited significant cytotoxicity against the three sentinel cancer cell lines, NCI-H460 (non-small cell lung cancer), MCF-7 (breast cancer), and SF-268 (CNS glioma). Bioassay-guided fractionation of this extract afforded three new cytochalasans, aspochalasins I−K (**1**−**3**), in addition to four known cytochalasans, aspochalasins C−E (**4**−**6**) and TMC-169 (**7**). Previous studies on *A. flavipes* strain TC 1446 collected in Japan have resulted in the isolation of the aspochalasin, TMC-169 (**7**).[7]



1: R_1= R_2=OH
1a: R_1= R_2=OAc
2: R_1=OH, R_2=H
2a: R_1=OAc, R_2=H



3: R_1=OMe, R_2=OH
3a: R_1=OMe, R_2=OAc
6: R_1= R_2=OH



4: R_1=H, R_2= R_3=OH
5: R_1= R_3=OH, R_2=H
7: R_1 or R_2=OH, R_3=H

Results and Discussion

The rhizosphere fungus, *Aspergillus flavipes*, was grown on potato dextrose agar (PDA) for 28 days. The culture was soaked in MeOH overnight and filtered. The filtrate was concentrated in vacuo and extracted sequentially with EtOAc and *n*-BuOH. Bioassay-guided fractionation of the cytotoxic EtOAc extract involving solvent−solvent partition, followed by Sephadex gel filtration and repeated silica gel chromatography, furnished seven cytotoxic compounds, three of which were new cytochalasans and were named aspochalasins I, J, and K. The structures of aspochalasins I, J, and K were established as **1**, **2**, and **3**, respectively, as described below, and the four known compounds were

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Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Spectral Data of Compounds **1**−**3** in $CDCl_3$

	1		**2**		**3**	
position	δ ^{1}H (mult., *J* in Hz)	δ ^{13}C (mult.)[a]	δ ^{1}H (mult., *J* in Hz)	δ ^{13}C (mult.)[a]	δ ^{1}H (mult., *J* in Hz)	δ ^{13}C (mult.)[a]
1		173.2 (s)		173.4 (s)		176.0 (s)
2	5.82 (br s)		5.80 (br s)		6.18 (br s)	
3	3.14 (dt, 9.9, 3.2)	51.8 (d)	3.14 (m)	51.8 (d)	3.13 (m)	51.0 (d)
4	2.92 (dd, 4.7, 3.1)	51.7 (d)	2.92 (m)	51.8 (d)	2.48 (m)	53.6 (d)
5	3.07 (br s)	34.2 (d)	3.07 (br s)	34.1 (d)	2.58 (m)	35.4 (d)
6		140.1 (s)		140.1 (s)		139.4 (s)
7	5.29 (br s)	124.2 (d)	5.29 (br s)	124.4 (d)	5.41 (br s)	125.8 (d)
8	3.80 (d, 10.9)	39.4 (d)	3.80 (m)	39.5 (d)	3.26 (d, 10.9)	43.5 (d)
9		88.1 (s)		88.1 (s)		67.7 (s)
10	1.50 (m), 1.32 (m)	48.5 (t)	1.50 (m), 1.30 (m)	48.4 (t)	1.15 (m)	48.8 (t)
11	1.20 (d, 7.3)	13.8 (q)	1.30 (d, 7.0)	13.8 (q)	1.17 (d, 7.2)	13.4 (q)
12	1.71 (br s)	19.7 (q)	1.70 (br s)	19.7 (q)	1.72 (d, 11.1)	19.8 (q)
13	6.20 (d, 10.9)	122.9 (d)	6.22 (d, 10.7)	122.9 (d)	6.00 (d, 11.0)	124.5 (d)
14		138.9 (s)		139.5 (s)		136.7 (s)
15	2.33 (m), 2.12 (m)	39.4 (t)	2.20 (m), 2.03 (m)	42.2 (t)	2.10 (m)	37.3 (t)
16	2.14 (m), 1.39 (m)	28.1 (t)	1.60 (m), 1.53 (m)	17.7 (t)	1.77 (m), 1.18 (m)	29.8 (t)
17	3.87 (d, 7.6)	78.0 (d)	2.10 (m), 1.76 (m)	38.0 (t)	3.86 (m)	72.5 (d)
18	4.53 (br s)	72.9 (d)	4.50 (m)	69.2 (d)	3.12 (m)	78.7 (d)
19	7.14 (dd, 15.2, 2.3)	150.2 (d)	7.33 (dd, 15.3, 2.6)	156.4 (d)	3.95 (m)	72.5 (d)
20	6.00 (dd, 15.2, 2.3)	120.1 (d)	5.91 (d, 15.3)	118.7 (d)	3.88 (d, 5.3), 2.01 (d, 16.2)	41.9 (t)
21		167.5 (s)		167.6 (s)		212.0 (s)
22	1.59 (m)	25.2 (d)	1.60 (m)	25.2 (d)	1.60 (m)	24.9 (d)
23	0.90 (d, 5.5)	21.4 (q)	0.86 (d, 5.6)	21.3 (q)	0.87 (d, 6.6)	21.5 (q)
24	0.91 (d, 5.5)	23.6 (q)	0.87 (d, 5.0)	23.6 (q)	0.89 (d, 6.6)	23.5 (q)
25	1.37 (br s)	15.2 (q)	1.50 (br s)	16.1 (q)	1.49 (br s)	15.8 (q)
OH	2.45 (br s)				2.94 (br s)	
OH	1.98 (br s)				2.86 (br s)	
OCH_3					3.45 (s)	57.4 (q)

[a] Multiplicities deduced from DEPT.

identified as aspochalasins C−E (**4**−**6**) and TMC-169 (**7**) by comparison of their spectral data with those reported in the literature.

Aspochalasin I (**1**) was isolated as a white powder. Its molecular formula was established as $C_{24}H_{35}NO_5$ from HRFABMS and ^{13}C NMR spectral data and indicated eight degrees of unsaturation. The IR spectrum had bands due to NH and/or OH (3390 cm^{-1}), an α,β-unsaturated ester/lactone carbonyl (1702 cm^{-1}), and an amide carbonyl (1685 cm^{-1}). The ^{1}H NMR spectrum (Table 1) showed the presence of an amide NH (δ 5.82, br s; exchangeable with D_2O), two OH groups (δ 2.45 and 1.98, br s; exchangeable with D_2O), three methyl doublets (δ 0.90, 0.91, and 1.20), two methyl singlets (δ 1.37 and 1.71), four olefinic protons [δ 5.29 (1H, br s), 6.00 (1H, dd), 6.20 (1H, d), and 7.14 (1H, dd)], and 11 methine and/or methylene protons. Acetylation afforded the diacetate (**1a**), confirming the presence of two OH groups in **1**. The ^{13}C NMR spectrum of **1** displayed signals for 24 carbon atoms and confirmed the presence of an α,β-unsaturated ester/lactone carbonyl (δ 167.5) and an amide carbonyl (δ 173.2) (Table 1). The combined analysis of ^{13}C NMR and edited HSQC spectra[10] revealed the presence of five methyl, three methylene, 11 methine, and three quaternary carbons in addition to the two carbonyls. Two of the quaternary carbons were olefinic [δ 138.9 (s) and 140.1 (s)], and one was oxygenated [δ 88.1 (s)]. Of the 11 methines, two were in the oxygenated region (δ 72.9 and 78.0) and four in the aromatic/olefinic region (δ 150.2, 124.4, 122.9, and 120.1). The above data accounted for five out of a total of eight degrees of unsaturation, suggesting the presence of three rings in the molecule. The ^{1}H−^{1}H correlations observed in the DQF-COSY spectrum suggested the presence of two partial structures, A ($C_{14}H_{19}O_4$) and B (C_8H_{15}), in **1** (see Figure 1), which were further confirmed by long-range ^{1}H−^{13}C correlations in the HMBC spectrum (Figure 2). The cross-peaks between δ_C 124.2 (C-7) and δ_H 3.07 (H-5); δ_C 34.2 (C-5) and δ_H 1.71 (H-12); and δ_C 140.1 (C-6) and δ_H 1.20 (H-11) and 2.92 (H-4) in the

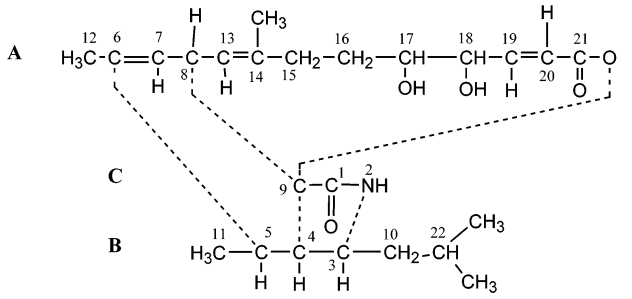

Figure 1. Partial structures of **1** based on DQF-COSY and their connectivities based on HMBC spectra.

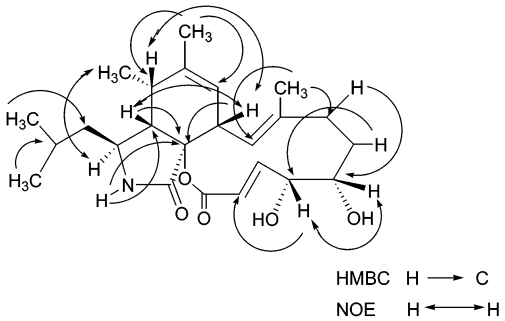

Figure 2. Selected key HMBC and NOE correlations of aspochalasin I (**1**).

HMBC spectrum established the connectivity between the olefinic carbon (C-6) in the partial structure A and a methine carbon (C-5) in the partial structure B. The remaining fragment of the molecule (C_2HNO) should contain the amide group and therefore is formulated as C−C(O)−NH (partial structure C; Figure 1). The chemical shift of the noncarbonyl quaternary carbon (C-9) of this fragment appearing at δ_C 88.1 suggested it to be oxygenated and therefore should be bonded to the oxygen at C-21 of fragment A, leading to a 12-membered macrocyclic

lactone ring. The HMBC correlations between δ_C 88.1 (C-9) and δ_H 3.07 (H-5) and 3.80 (H-8) suggested C-9 to be a common point of attachment of the partial structures A and B. Finally, the cross-peaks between δ_C 88.1 (C-9) and 51.7 (C-4) with δ_H 5.82 (NH) in the HMBC spectrum suggested the attachment of the amide nitrogen to C-3 of the fragment B, leading to an aspochalasin-type structure for **1**, analogous to previously known phomasins A and B.[8]

The relative stereochemistry of **1** was determined with the help of ^{1}H NMR coupling constants, pulse field gradient 1D NOE experiments, and comparison of NMR data with those of known aspochalasins. Thus, the large coupling constant (15.2 Hz) between H-19 and H-20 suggested an *E* configuration for the C-19(20) double bond. A pulse field gradient 1D NOE experiment showed an enhancement of ^{1}H signals at δ 3.07 (H-5) and 2.92 (H-4) when the ^{1}H signal at δ 3.80 (H-8) was irradiated, suggesting that these protons were on the same side of the cyclohexane ring. The enhancement of the methyl doublet at δ 1.20 (H-11) on irradiation of the signal at δ 3.14 (H-3) indicated that the 2-methylpropyl group at C-3 has a β-orientation and that C-5-Me and H-3 have an orientation opposite of that of H-4. When the signal at δ 4.53 (H-18) was irradiated, the signal at δ 3.87 (H-17) showed an Overhauser effect, suggesting the vicinal OH groups at C-17 and C-18 to be of *cis*-orientation. On the basis of the above spectral data, the structure of aspochalasin I was assigned as **1**, which differs from that of phomacin A only by the absence of a methyl group at C-16 in **1**. Comparison of ^{1}H and ^{13}C NMR data including ^{1}H NMR coupling constants of **1** and phomacin A,[8] while confirming the structure proposed for aspochalasin I, suggested that both compounds have the same relative stereochemistry. It is noteworthy that in all cytochalasans isolated thus far the stereochemistry of the cyclohexane and isoindole moieties are the same. Thus the structure of aspochalasin I was assigned as 17α,18α-dihydroxy-10-isopropyl-14-methyl[12]cytochalasa-6,13,19-trien-1-one-21-(9)-lactone (**1**).

The molecular formula of aspochalasin J (**2**) was determined as $C_{24}H_{35}NO_4$ from HRFABMS and ^{13}C NMR data. ^{1}H and ^{13}C NMR spectra (see Table 1) showed very close resemblance to those of **1**, suggesting that aspochalasin J had the same carbon skeleton as aspochalasin I (**1**). The ^{1}H and ^{13}C NMR data, in addition to providing evidence for the presence of a macrocyclic lactone ring in **2**, showed the presence of three double bonds (δ_C 156.4, 140.1, 139.5, 124.4, 122.9, and 118.7), five methyl groups (δ_C 13.8, 16.1, 19.7, 21.3, and 23.6), and an amide carbonyl (δ_C 173.4). However, unlike **1**, compound **2** had only one oxygenated methine (δ_H 4.50; δ_C 69.2) bearing an OH. The presence of OH in **2** was further confirmed by the MS fragment at *m/z* 384 due to the loss of a molecule of H_2O from M^+ and acetylation of **2** to afford the monoacetyl derivative **2a**. The OH group was located at C-18 due to the presence of a cross-peak between δ_H 4.50 (H-18) and 7.33 (H-19) in its DQF-COSY spectrum. Although the orientation of the OH at C-18 was not determined due to the limited availability of **2**, comparison of ^{1}H and ^{13}C chemical shifts for H-18 and C-18 with those reported[8] for phomacins B and C suggested that the OH in **2** had the same α-orientation. On the basis of the above data aspochalasin J was identified as 18α-hydroxy-10-isopropyl-14-methyl[12]cytochalasa-6,13,19-trien-1-one-21(9)-lactone (**2**).

Aspochalasin K (**3**) was found to have the molecular formula $C_{25}H_{39}NO_5$ by HRFABMS. Its ^{1}H and ^{13}C NMR spectra were similar to those of **1** except for the absence of the α,β-unsaturated lactone moiety (Table 1). Instead, these spectra showed the presence of a macrocyclic ketone (δ_C 212.0) and an OCH_3 substituent [δ_C 57.4 (q); δ_H 3.45 (3H)]. The remaining two oxygen atoms were suspected to belong to two OH groups, and this was confirmed by conversion of aspochalasin K into its diacetate (**3a**) with Ac_2O/pyridine. Careful analysis of the DQF-COSY spectrum and comparison of ^{1}H NMR spectral data indicated that the three oxygenated methines were at the same position as in aspochalasin C (**4**),[5] another aspochalasan encountered in this study. The presence of a cross-peak at δ_C 78.7 (C-18)/δ_H 3.45 in the HMBC spectrum of **3** suggested that the OCH_3 group is located at C-18. Analysis of coupling constants and NOE data did not permit the definitive assignment of the stereochemistry of the substituents at C-17, C-18, and C-19. Aspochalasin K was therefore tentatively identified as 17,19-dihydroxy-18-methoxy-10-isopropyl-14-methyl[12]cytochalasa-6,13-dien-1,21-dione (**3**).

Table 2. Cytotoxicities of Compounds **1**−**7** against a Panel of Tumor Cell Lines[a]

	cell line[b]		
compound	NCI-H460	MCF-7	SF-268
1	22.1	33.4	19.9
2	55.2	14.9	13.4
3	14.3	13.1	19.9
4	5.0	7.7	3.9
5	3.4	5.3	5.0
6	31.5	30.4	52.6
7	3.5	5.0	9.5
Taxol	9.5	11.2	21.7

[a] Results are expressed as IC_{50} values in μM except for Taxol, which is in nM. [b] Key: NCI-H460 = human non-small cell lung cancer; MCF-7 = human breast cancer; SF-268 = human CNS cancer (glioma).

On the basis of their EIMS and ^{1}H and/or ^{13}C NMR spectral data compounds **4**−**7** were identified respectively as aspochalasins C[3], D,[4] and E[5] and TMC-169.[7]

All aspochalasins (**1**−**7**) were evaluated for their in vitro cytotoxicity against a panel of three sentinel cancer cell lines, NCI-H460 (non-small cell lung), MCF-7 (breast), and SF-268 (CNS glioma). Cells were treated with test compounds for 48 h in RPMI 1640 media supplemented with 10% fetal bovine serum, and cell viability was evaluated by MTT assay.[11] As shown in Table 2, aspochalasin C (**4**), aspochalasin D (**5**), and TMC-169 (**7**), all bearing an α,β-unsaturated ketone moiety, were found to be considerably more active than aspochalasins I (**1**) and J (**2**), having an α,β-unsaturated lactone moiety, and aspochalasins K (**3**) and E (**6**), with only one carbonyl group, suggesting the importance of an electrophilic α,β-unsaturated carbonyl moiety for the cytotoxic activity of these cytochalasins.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-John's melting point apparatus and are uncorrected. Optical rotations were measured with a Jasco Dip-370 polarimeter using $CHCl_3$ as solvent. IR spectra for KBr disks were recorded on a Shimadzu FTIR-8300 spectrometer. 1D and 2D NMR spectra were recorded in $CDCl_3$ with a Bruker DRX-500 instrument at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR using residual $CHCl_3$ as internal standard. The chemical shift values (δ) are given in parts per million (ppm), and the coupling constants are in Hz. Low-resolution and high-resolution MS were recorded respectively on Shimadzu LCMS-8000 QPα and JEOL HX110A spectrometers.

Fungal Isolation, Identification, and Cultivation. The fungal strain was isolated from the rhizosphere of the turpentine brush (*Ericameria laricifolia* Nutt.) growing in Tumamoc Hill in Tucson, Arizona, and was identified by D.B. (Depart-

ment of Plant Pathology, University of Arizona) as *Aspergillus flavipes* by analysis of the ITS regions of the ribosomal DNA as described previously.[1] Excised roots of *E. laricifolia* (1 cm long sections; ca. 5 g) were placed in 5 mL of phosphate-buffered saline (PBS, 0.1 M, pH = 7.4), and microorganisms were detached from the roots by vortexing and sonication. A serial dilution of the suspension was placed on potato dextrose agar (PDA, Difco, Plymouth, MN) supplemented with chloramphenicol and streptomycin. After 4 days of incubation at 25 °C, single colonies were transferred to water agar containing the same antibiotics, and after 3 days a pure culture of *A. flavipes* was obtained by hyphal tipping. The strain is deposited in the Department of Plant Pathology and Southwest Center for Natural Products Research and Commercialization of the University of Arizona microbial culture collection under the code name Erl-1-F17. The organism was subcultured using Petri dishes with PDA, and for long-term storage isolates were subcultured on PDA slants, overlaid with 40% glycerol, and stored at −80 °C. For isolation of bioactive compounds the fungus was cultured in 40 T-flasks (500 mL) each containing 135 mL of PDA coated on five sides of the T-flask (total surface area ca. 460 cm^2) for 28 days at 27 °C.

Extraction and Isolation. Methanol (200 mL/T-flask) was added to all 40 T-flasks and allowed to soak overnight at room temperature, and the resulting extract was filtered through Whatman No. 1 filter paper and a layer of Celite 545. The filtrate was concentrated to one-fourth of its original volume and extracted sequentially with EtOAc (5 × 500 mL) and *n*-BuOH (5 × 300 mL). Evaporation under reduced pressure afforded EtOAc (3.85 g) and *n*-BuOH (0.50 g) extracts, of which the former was found to be cytotoxic. The cytotoxic EtOAc extact (3.50 g) was partitioned between hexane and 80% aqueous MeOH, and the cytotoxic aqueous MeOH fraction was diluted to 60% aqueous MeOH by the addition of water and extracted with $CHCl_3$. Evaporation of $CHCl_3$ under reduced pressure yielded a pale brown semisolid (3.09 g), which was found to be cytotoxic. A portion (500 mg) of this was subjected to gel permeation chromatography on a column of Sephadex LH-20 (15.0 g) made up in hexane/CH_2Cl_2 (1:4) and eluted with 300 mL each of hexane/CH_2Cl_2 (1:4), CH_2Cl_2/acetone (3:2), CH_2Cl_2/acetone (1:4), CH_2Cl_2/MeOH (1:1), and finally with MeOH. One hundred and fifty fractions (10 mL each) were collected and pooled based on their TLC patterns to yield 20 combined fractions (F1−F20), of which fractions F3−F8 were found to be cytotoxic. Bioactive fractions F5−F8 were combined and subjected to further fractionation by silica gel column chromatography with CH_2Cl_2/MeOH (94:6) as eluting solvent, and the cytotoxic fractions were separated by silica gel column chromatography using a gradient of 2-propanol (3% to 10%) in hexane followed by preparative TLC to afford **1** (2.6 mg), **4** (5.6 mg), **5** (2.0 mg), and **6** (34.2 mg). Combined bioactive fractions F3−F4 on silica gel chromatography using a gradient of 2-propanol (4% to 10%) in hexane followed by preparative TLC gave **7** (1.5 mg), **2** (1.1 mg), and **3** (13.5 mg).

Cytoxicity Bioassays. The tetrazolium-based colorometric assay (MTT assay)[11] was used for the in vitro assay of cytotoxicity to human nonsmall cell lung carcinoma (NCI-H460), human breast carcinoma (MCF-7) and human glioma (SF-268) cells as previously reported.[1]

Aspochalasin I (1): white solid; mp 136−138 °C; $[\alpha]^{27}_D$ −166.60° (*c* 0.2, $CHCl_3$); IR ν_{max} 3390, 3240, 2957, 2920, 2851, 1702, 1685, 1450, 1238 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR data (125 MHz), see Table 1; MS *m*/*z* 418 ($[M + H]^+$, in +APCI mode), 400, 384, 316; HRFABMS *m*/*z* 418.2585 $[M + H]^+$ (calcd for $C_{24}H_{36}NO_5$, 418.2593).

Diacetyl Aspochalasin I (1a). Aspochalasin I (0.8 mg) in dry pyridine (0.1 mL) was treated with Ac_2O (0.05 mL) for 24 h at room temperature. Usual workup and preparative TLC afforded the acetyl derivative (**1a**, 0.6 mg): ^1H NMR (500 MHz, $CDCl_3$) δ 7.19 (1H, dd, *J* = 1 5.3, 3.0 Hz, H-19), 6.31 (1H, d, *J* = 10.0 Hz, H-13), 5.82 (1H, d, *J* = 15.3 Hz, H-20), 5.33 (1H, d, *J* = 6.0 Hz, H-7), 5.86 (1H, d, *J* = 10.5 Hz, NH), 4.96 (1H, d, *J* = 8.0 Hz, H-18), 4.25 (1H, dd, *J* = 8.0, 5.0 Hz, H-17), 3.80 (1H, m, H-8), 3.18 (1H, d, *J* = 10.0 Hz, H-3), 3.07 (1H, m, H-5), 2.94 (1H, dd, *J* = 4.5, 3.5 Hz, H-4), 2.33 (1H, m, H-15a), 2.22 (3H, s, Ac), 2.14 (1H, m H-16a), 2.12 (1H, m, H-15b), 2.07 (3H, s, Ac), 1.71 (3H, s, H-12), 1.53 (1H, m, H-10a), 1.39 (1H, m, H-16b), 1.37 (3H, s, H-25), 1.32 (1H, m, H-10b), 1.20 (3H, d, *J* = 7.2 Hz, H-11), 0.90 (3H, d, *J* = 6.3 Hz, H-23), 0.91 (3H, d, *J* = 6.3 Hz, H-24); MS *m*/*z* 502 ($[M + H]^+$, in +APCI mode), 500 ($[M - H]^-$, in −APCI mode).

Aspochalasin J (2): white solid; IR ν_{max} 3241, 2957, 2921, 2850, 1709, 1682, 1452, 1237 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz) data, see Table 1; MS *m*/*z* 402 ($[M + H]^+$, in +APCI mode), 384; 400 ($[M - H]^-$, in −APCI mode); HRFABMS *m*/*z* 402.2638 $[M + H]^+$ (calcd for $C_{24}H_{36}NO_4$, 402.2644).

Acetyl Aspochalasin J (2a). Aspochalasin J (0.3 mg) in dry pyridine (0.05 mL) was treated with Ac_2O (0.05 mL) for 24 h at room temperature. Usual workup and preparative TLC afforded the acetyl derivative (**2a**, 0.2 mg): MS *m*/*z* 444 ($[M + H]^+$, in +APCI mode), 442 ($[M - H]^-$, in −APCI mode).

Aspochalasin K (3): white solid; mp 185−186 °C; $[\alpha]^{27}_D$ −81.42° (*c* 1.0, $CHCl_3$); IR ν_{max} 3470, 3220, 2957, 2921, 2850, 1695, 1451, 1100 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz) data, see Table 1; MS *m*/*z* 434 ($[M + H]^+$, in +APCI mode), 416, 398, 384; *m*/*z* 432 ($[M - H]^-$ in −APCI mode), 414, 400, 376; HRFABMS *m*/*z* 434.2906 $[M + H]^+$ (calcd for $C_{25}H_{40}NO_5$, 434.2906).

Diacetyl Aspochalasin K (3a). Aspochalasin K (1.0 mg) in dry pyridine (0.1 mL) was treated with Ac_2O (0.05 mL) for 24 h at room temperature. Usual workup and preparative TLC afforded the acetyl derivative (**3a**, 0.8 mg): ^1H NMR (500 MHz, $CDCl_3$) δ 6.21 (1H, d, *J* = 11.0 Hz, H-13), 5.70 (1H, br s, NH), 5.44 (1H, br s, H-7), 5.16 (1H, d, *J* = 10.0 Hz, H-19), 5.08 (1H, br s, H-17), 4.36 (1H, br d, *J* = 13.5 Hz, H-20a), 3.45 (3H, s, OMe), 3.20 (1H, d, *J* = 10.5 Hz, H-8), 3.14 (2H, m, H-3, H-18), 2.57 (1H, m, H-5), 2.48 (1H, m, H-4), 2.10 (2H, m, H-15), 2.09 (3H, s, Ac), 2.05 (1H, m, H-20b), 1.96 (3H, s, Ac), 1.77 (1H, m, H-16a), 1.73 (3H, s, H-12), 1.60 (1H, m, H-22), 1.55 (3H, s, H-25), 1.35 (2H, m, H-10), 1.18 (1H, m, H-16b), 1.18 (3H, d, *J* = 5.7 Hz, H-11), 0.89 (3H, d, *J* = 6.0 Hz, H-23), 0.88 (3H, d, *J* = 6.0 Hz, H-24); MS *m*/*z* 518 ($[M + H]^+$, in +APCI), 516 ($[M - H]^-$, in −APCI).

Aspochalasin C (4): white amorphous solid; mp 126−128 °C; $[\alpha]^{27}_D$ −76.5° (*c* 1.0, $CHCl_3$) {lit.[3] $[\alpha]^{25}_D$ −86° (*c* 1.37, $CHCl_3$)}; spectral data (^1H NMR, EIMS) consistent with literature values.[3]

Aspochalasin D (5): white amorphous powder; $[\alpha]^{27}_D$ −108.0° (*c* 0.2, $CHCl_3$) {lit.[4] $[\alpha]^{25}_D$ −81.0° (*c* 1.43, EtOH)}; spectral data (^1H and ^{13}C NMR, EIMS) consistent with literature values.[4]

Aspochalasin E (6): white amorphous powder; mp 129−132 °C [lit.,[5] 129−131 °C]; $[\alpha]^{27}_D$ −32.4° (*c* 1.0, $CHCl_3$) {lit.[5] $[\alpha]^{24}_D$ − 52° (*c* 0.5, $CHCl_3$)}; spectral data (^1H and ^{13}C NMR, EIMS) consistent with literature values.[5]

TMC-169 (7): white amorphous solid; mp 112−114 °C [lit.[7] 103−105 °C]; $[\alpha]^{27}_D$ −47.8° (*c* 1.0, $CHCl_3$) {lit.[7] $[\alpha]^{27}_D$ −50° (*c* 0.31, MeOH)}; spectral data (^1H and ^{13}C NMR and EIMS) consistent with literature values.[7]

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