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New Cytochalasins from the Fungus *Xylaria hypoxylon*

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Abstract: *Six new cytochalasins together with the known compounds cytochalasins Q, 19,20-epoxy Q and R have been isolated from the fermentation broth of the fungus Xylaria hypoxylon. The structures of the new compounds were established by extensive NMR inverse spectrometry and FAB-MS experiments.* © 1997 Elsevier Science Ltd.

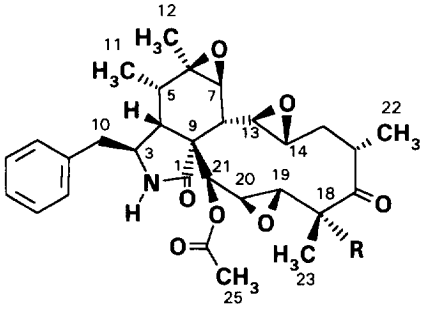
INTRODUCTION

In the course of our screening for new biologically active compounds, a fungal strain identified as *Xylaria hypoxylon* was found to be a rich source of several new cytochalasins. Cytochalasins are fungal metabolites which have been isolated from different genera of fungi such as *Phomopsis*, *Chaetomium*, *Hypoxylon* and most recently from *Xylaria* and *Daldinia*[1-4]. Cytochalasins exhibit several biological activities including marked cytostatic effects on mammalian cells in tissue culture [4,5], inhibition of HIV-1 protease[2], antibiotic and antitumour activity[5,6], etc. In this paper, we report the isolation and structural elucidation of six new cytochalasins produced by a fungus belonging to the genus *Xylaria*.

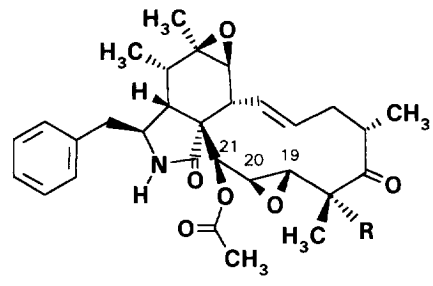
RESULTS AND DISCUSSION

A fungus, taxonomically classified as *Xylaria hypoxylon* (L.)[7], was isolated from a soil sample containing decayed wood chips collected at Tikal, Guatemala. This *X. hypoxylon* was grown in beef extract liquid culture. After nine days of incubation the fermentation broth was centrifuged and the supernatant was extracted with EtOAc. This crude extract was fractionated on a silica gel column giving two fractions composed mainly of a complex mixture of compounds, which could be identified as cytochalasins from characteristic ^{1}H-NMR signals. The purification of these fractions was carried out by extensive reverse-phase HPLC to obtain the pure compounds **1-9**.

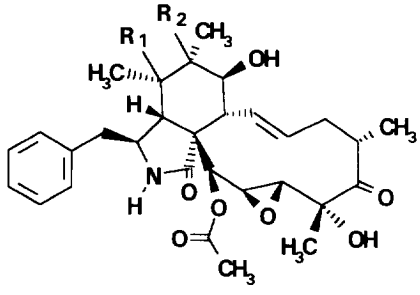
Compounds **3**, **4** (the major component of the mixture) and **6** were identified as cytochalasin R, 19,20-epoxycytochalasin Q and cytochalasin Q respectively by comparison of their spectroscopic data with those reported in the literature[2,3].



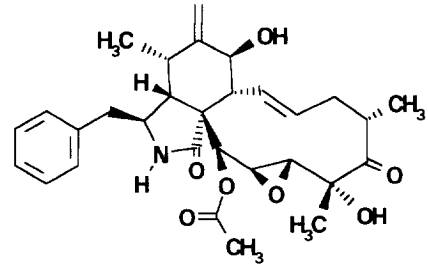
1 R = OH
2 R = H
3 19-20 double bond; R = OH



4 R = OH
5 R = H
6 19-20 double bond; R = OH



7 R1 = R2 = epoxide
8 R1 = R2 = double bond



9

Figure 1. Structure of compounds **1**-**9**.

Compound **1** was crystallized from $CHCl_3$ as a white microcrystalline solid. Its FAB-MS (positive ion mode) showed a molecular ion peak at m/z 540 $[M+H]^+$, 16 units larger than that of **3**, indicating the presence of an extra oxygen atom and thus a molecular formula $C_{30}H_{37}NO_8$. The FAB-MS of these two compounds showed the same fragmentation pattern with a base peak at m/z 91 (C_7H_7) and a fragment at m/z 43 (C_2H_3O) characteristic of benzyl and acetyl groups respectively. The IR spectrum of **1** presented absorbances of ester (at 1740 cm^{-1}) and amide (at 1690 cm^{-1}). ^1H and ^{13}C-NMR data (see Tables 1 and 2) confirmed the presence of these functions. Comparison of their ^{13}C NMR spectra showed the presence of the same functionalities except for the C19-C20 double bond that is epoxidized in **1**. This was confirmed by the correlation observed in the HSQC experiment between the proton signals at δ_H 3.33 (d, J=1.7 Hz) and 3.63 (dd, J= 2.0 and 1.4 Hz) with the carbon signals at δ_C 57.86 (d) and 52.63 (d) respectively. DQF-COSY and HOHAHA experiments

TABLE 1. ^{1}H NMR spectral data for cytochalasins **1-2** and **4-9** recorded in $CDCl_3$.

H	1	2	4	5	6	7	8	9
NH	5.91 br s	5.65 br s	5.72 br s	5.68 br s	5.69 br s	5.71 br s	5.56 br s	5.46 br s
3	3.65 m	3.65 m	3.57 br t (6.8)	3.57 br dt (7.2,1.8)	3.55 br t (7.3)	3.58 m	3.33 dd (8.2, 6.7)	3.24 m
4	2.26 dd (4.3, 2.9)	2.07 m	2.19 dd (5.3, 2.0)	2.10 m	2.08 dd (5.7, 2.3)	2.55 d (2.4)	2.52 br s	2.25 dd (5.2, 3.3)
5	1.48 m	1.54 m	1.60 m	1.61 dq (7.3, 5.6)	1.69 dq (7.3, 5.7)			2.62 m
7	2.96 d (5.6)	3.01 d (5.1)	2.69 d, (5.5)	2.71 d (5.7)	2.70 d (5.6)	3.49 dd (10.4, 1.5)	3.78 br d (9.8)	3.81 br d (10.1)
8	1.38 dd (8.5, 5.6)	1.62 dd (7.1, 5.2)	2.31 dd (10.0, 5.6)	2.25 dq (8.7, 6.9)	2.50 dd (10.0, 5.6)	2.90 dd (10.7,10.4)	2.26 dd (10.2, 9.8)	2.62 m
10	2.87 dd (13.5, 5.8)	2.90 dd (13.5, 4.8)	2.82 d (7.3)	2.80 m	2.75 d (7.2)	3.05 dd (13.5, 5.4)	3.06 dd (13.5, 6.0)	2.85 dd (13.4, 5.0)
	2.81 dd (13.5, 8.0)	2.69 m				2.89 dd (13.5, 7.9)	2.99 dd (13.5, 9.2)	2.73 dd (13.4, 9.1)
11	0.83 d (7.3)	0.96 d (7.3)	0.83 d (7.4)	0.84 d (7.3)	0.96 d (7.3)	1.36 s	1.41 s	0.89 d (6.9)
12	1.20 s	1.26 s	1.16 s	1.18 s	1.16 s	1.19 s	1.65 s	5.27 br s, 5.06 br s
13	3.62 dd (8.5, 1.8)	3.75 dd (7.0, 2.2)	6.01 dd (15.6,10.0)	6.25 dd (15.3,10.0)	5.81 dd (15.7,10.0)	6.06 dd (15.6, 10.7)	6.13 dd (15.7, 10.2)	5.89 dd (15.5, 9.8)
14	2.84 m	2.69 m	5.61 ddd (15.7, 10.1, 6.0)	5.54 ddd (15.7, 10.8, 5.1)	5.23 ddd (15.7, 10.8,5.2)	5.70 ddd (15.6, 10.8, 5.7)	5.70 ddd (15.8, 9.9, 6.1)	5.69 ddd (15.5, 9.9, 5,8)
15	2.23 ddd (15.0,1.5,1.5)	2.07 m	2.60 ddd (12.9, 12.1, 10.1)	2.47 ddd (13.5, 11.3, 10.8)	2.45 ddd (11.6, 11.1,11.1)	2.61 ddd (12.0, 11.5, 10.8)	2.67 ddd (12.6, 10.1, 10.0)	2.62 m
	1.75 ddd (15.1, 12.1, 9.7)	1.84 ddd (15.3, 12.2, 6.8)	2.07 dd (12.9, 5.2)	2.15 m	1.97 ddt (13.0, 5.3, 1.0)	2.09 dd (11.5, 5.7)	2.09 m	2.09 m
16	3.22 m	2.86 m	3.19 ddq (12.1, 6.7, 2.2)	2.96 ddq (13.5, 6.5, 3.7)	2.71 m	3.19 m	3.22 ddq (10.8, 6.7, 1.2)	3.22 m
18		2.54 dq (8.9, 6.8)		2.25 dq (8.7, 6.9)				
19	3.33 d (1.7)	3.03 dd (7.2, 2.0)	3.14 d (2.0)	2.92 dd (8.7, 1.9)	6.06 dd (15.7, 2.5)	3.15 d (1.9)	3.19 d (2.0)	3.14 d (1.9)
20	3.63 dd (2, 1.4)	3.89 dd (2.0, 1.4)	3.51 dd (2.0, 1.5)	3.53 dd (2, 0.8)	5.09 dd (15.7, 2.4)	3.35 dd (1.9, 1.2)	3.43 dd (2.0, 0.8)	3.53 dd (1.9, 0.8)
21	5.58 s	5.45 s	5.62 s	5.54 s	5.77 dd (2.5, 2.4)	5.50 br s	5.74 br s	5.51 br s
22	1.22 d (6.7)	1.15 d (6.9)	1.18 d (6.7)	1.11 d (6.9)	1.17 d (6.9)	1.18 d (7.3)	1.18 d (6.7)	1.18 d (6.6)
23	1.57 s	1.28 d (6.7)	1.51 s	1.27 d (7.0)	1.47 s	1.51 s	1.50 s	1.53 s
25	2.06 s	2.06 s	2.10 s	2.10 s	2.20 s	2.17 s	2.14 s	2.14 s
2',6'	7.33 m	7.35 m	7.34 m	7.34 m	7.31 m	7.33 m	7.31 m	7.32 m
3',5'	7.19 m	7.15 m	7.17 m	7.16 m	7.14 m	7.19 m	7.19 m	7.15 m
4'	7.26 m	7.15 m	7.17 m	7.16 m	7.24 m	7.25 m	7.25 m	7.24 m

TABLE 2. ^{13}C NMR spectral data of **1-9** recorded in $CDCl_3$.

C	1	2	3	4	5	6	7	8	9
1	174.12 s	174.23 s	174.18 s	174.24 s	174.51 s	174.41 s	173.79 s	174.03 s	173.43 s
3	54.94 d	54.84 d	54.32 d	54.43 d	54.31 d	54.03 d	56.54 s	60.62 d	53.90 d
4	52.55 d	51.94 d	50.98 d	51.39 d	50.96 d	50.69 d	49.29 d	50.66 d	50.71 d
5	36.67 d	36.66 d	36.84 d	36.71 d	36.68 d	36.82 d	64.05 s	126.53 s	32.56 d
6	55.93 s	55.89 s	55.74 s	57.22 s	57.16 s	57.03 s	63.03 s	131.29 s	147.35 s
7	61.31 d	59.53 d	61.23 d	62.41 d	62.45 d	62.54 d	68.87 d	68.01 d	69.98 d
8	43.66 d	42.01 d	43.95 d	44.77 d	44.54 d	45.13 d	43.39 d	49.16 d	46.54 d
9	53.44 s	52.87 s	54.18 s	54.47 s	53.99 s	55.04 s	53.41 s	51.39 s	52.45 s
10	45.73 t	45.39 t	45.94 t	45.80 t	45.88 t	45.91 t	44.80 t	44.69 t	45.18 t
11	12.53 q	13.61 q	12.56 q	12.42 q	12.68 q	12.55 q	14.10 q	13.93 q	13.49 q
12	19.80 q	19.95 q	19.48 q	19.57 q	19.68 q	19.30 q	19.59 q	17.12 q	114.44 t
13	57.47 d	57.68 d	58.20 d	131.38 d	131.36 d	131.84 d	130.38 d	133.89 d	131.17 d
14	59.65 d	57.57 d	61.49 d	131.12 d	130.77 d	131.79 d	135.04 d	131.61 d	133.47 d
15	38.63 t	36.02 t	37.88 t	36.71 t	37.52 t	37.91 t	37.65 t	37.36 t	37.40 t
16	37.53 d	45.97 d	37.71 d	41. 79 d	43.63 d	42.27 d	41.70 d	41.81 d	41.89 d
17	214.24 s	214.93 s	212.50 s	215.40 s	215.91 s	210.39 s	215.15 s	215.22 s	215.28 s
18	76.37 s	44.86 d	77.57 s	76.31 s	50.56 d	77.74 s	76.31 s	76.29 s	76.32 s
19	57.86 d	58.00 d	131.41 d	59.88 d	58.56 d	130.85 d	59.57 d	59.86 d	59.63 d
20	52.63 d	57.51 d	129.07 d	52.79 d	57.39 d	127.66 s	52.79 d	53.01 d	52.72 d
21	72.92 d	74.12 d	75.30 d	72.70 d	72.82 d	75.85 d	72.50 d	71.93 d	74.06 d
22	19.56 q	17.57 q	20.29 q	19.07 q	18.64 q	19.57 q	19.11 q	19.18 q	19.17 q
23	21.86 q	15.64 q	24.24 q	21.83 q	14.77 q	24.22 q	21.75 q	21.86 q	21.86 q
24	169.44 s	169.80 s	169.55 s	169.81 s	170.02 s	170.66 s	170.30 s	170.05 s	169.82 s
25	20.56 q	20.49 q	20.69 q	20.61 q	20.58 q	20.76 q	20.84 q	20.76 q	20.68 q
1'	136.53 s	136.79 s	136.79 s	136.79 s	136.90 s	136.97 s	136.74 s	137.46 s	137.11 s
2',6'	129.25 d	129.17 d	129.07 d	129.20 d	129.14 d	129.14 d	129.14 d	129.08 d	129.14 d
3',5'	129.06 d	128.98 d	129.07 d	128.99 d	129.02 d	128.97 d	129.11 d	128.92 d	128.97 d
4'	127.29 d	127.37 d	127.25 d	127.16 d	127.18 d	127.37 d	127.33 d	127.02 d	127.13 d

revealed the coupling between the above mentioned protons with the methine proton H-21 at δ_H 5.58 (s). The relative stereochemistry of all chiral centers of the molecule was established by comparison of the chemical shifts and coupling constants described for the corresponding centres in cytochalasins Q and R, and the configuration of the 19,20-epoxide function by the cross peaks observed in the ROESY spectrum. The correlations for H-25/H-19, H-14/H-19, H-16/H-19, H-18/H-19, H-18/H-16, H-14/H-16 on one side of the molecule, together with the cross peak for H-20/OH-18 on the other side, showed that the configuration of the 19,20-epoxide is trans as shown in figure 1. Thus, compound **1** was identified as 21-acetoxy-6,7,13,14,19,20-triepoxy-18-hydroxy-16,18-dimethyl-10-phenyl[11]cytochalas-1,17-dione which we have abbreviated to 19,20-epoxycytochalasin R.

The FAB-MS spectrum of compound **2** gave the molecular ion peak at m/z 524 $[M+H]^+$ indicating a molecular formula $C_{30}H_{37}NO_7$. This formula is 16 mass units smaller than that of **1**, suggesting the absence of an oxygen. The ^1H and ^{13}C-NMR spectra of **2** were very similar to those of compound **3**, with the exception of the presence of a methine proton at δ_H 2.54 (dq, J=8.9 and 6.8 Hz) which correlated with a tertiary carbon at δ_C 44.86 in the HSQC spectrum indicating the absence of the -OH group at C-18. Thus, the structure of compound **2** was established as 18-deoxy-19,20-epoxycytochalasin R.

Compound **5** was crystallized from MeOH, and its FAB-MS shows a molecular ion peak at m/z 508 $[M+H]^+$ and thus a molecular formula $C_{30}H_{37}NO_6$, one oxygen atom less than in **4**. Its ^1H-NMR spectrum revealed a signal at δ 2.25 ppm (dq, J=8.7 and 6.9 Hz) which showed correlations with the proton signals at δ 2.92 (H-19) and 1.27 ppm (H-23) respectively in the DQF-COSY spectrum. The presence of the tertiary carbon at δ 50.56 ppm and the lack of a signal for a quaternary carbon around 76.0-78.0 ppm in the ^{13}C-NMR spectrum allowed us to locate the absence of the -OH group at C-18. The chemical shifts for the carbons C-18, C-20 and C-23 (see table 2) are in agreement with the values observed for 18-deoxy-19,20-epoxycytochalasin R (**2**). The relative stereochemistry was determined by ROESY experiments and it was found to be the same as that of 19,20-epoxycytochalasin Q (**2**). In this way, the structure of compound **5** was assigned as 18-deoxy-19,20-epoxycytochalasin Q.

Further purification of the ethyl acetate supernatant extract led to the isolation of compound **7**. Its FAB-MS gave a molecular ion peak $[M+H]^+$ at m/z 540 in agreement with the molecular formula $C_{30}H_{37}NO_8$. This compound is isomeric with 19,20-epoxycytochalasin R **1** although it was much more polar in the HPLC chromatogram. Comparison of ^1H and ^{13}C-NMR spectra of **1** and **7** suggested the presence in **7** of an additional hydroxyl group and a double bond in place of the 13,14-epoxide function. The combined results of the DQF-COSY and HOHAHA spectra confirmed this by the correlations observed between the protons H-16 at δ 3.19 ppm (m), H-15 at δ 2.61 (ddd, J= 12.0, 11.5 and 10.8 Hz) and 2.09 ppm (dd, J= 11.5 and 5.7 Hz) with the olefinic protons at δ 6.06 (dd, J= 15.6 and 10.7 Hz) and 5.70 ppm (ddd, J= 15.6, 10.8 and 5.7 Hz) which were assigned to the Δ^{13} double bond. The location of the secondary hydroxyl group at C-7 was established on the basis of HOHAHA and HSQC correlation data. HOHAHA experiments showed the expected coupling patterns of H-8 and H-13 and confirmed that C-7 is a tertiary carbon. The presence of the epoxide function at the 5,6 position was confirmed by the cross-peaks detected in the HMBC experiment

between the quaternary carbons C-5 at δ 64.0 ppm and the protons H-4 and H-11 and between C-6 at δ 63.03 and the protons H-7 and H-12. A precedent for an epoxide function in this position is found in cytochalasin N previously isolated from the fungus *Hypoxylon terricola*[2] establishing **7** as 19,20-epoxycytochalasin N.

Another metabolite isolated from the same extract was compound **8**, obtained as a white powder. Its molecular formula was established as $C_{30}H_{37}NO_7$ by the molecular ion peak observed at m/z 524 $[M+H]^+$ in its FAB-MS, 16 mass units smaller than that of compound **7**. Comparison of their ^1H and ^{13}C-NMR spectra suggested the presence of a double bond at the 5,6 position. So compound **8** was assigned the structure of 19,20-epoxycytochalasin C.

Finally, compound **9** was crystallized from methanol as a microcrystalline white solid. The molecular formula $C_{30}H_{37}NO_7$ was deduced from its positive ion mode FAB-MS which showed a molecular ion peak at m/z 524. This compound is isomeric with compounds **2-4** and **8**. Its ^1H and ^{13}C-NMR data were very similar to those of compound **8**, except for the presence of two extra signals at δ 5.27 (1H, br s) and 5.06 ppm (1H, br s) which correlate with a signal at δ 114.44 ppm in the ^{13}C NMR spectrum, and the lack of the signal corresponding to the C-12 methyl group indicating an exo-methylene group at C-20(12). The chemical shifts and coupling constants around the cyclohexane ring are in agreement with those reported for cytochalasin D. Therefore, the structure of compound **9** is 19,20-epoxycytochalasin D or 21-acetylengleromycin.[8]

Studies on the biological activities of these compounds are in progress and will be published elsewhere.

EXPERIMENTAL

General Methods. Optical rotations were determined in $CHCl_3$ solutions on a JASCO DIP-370 polarimeter. IR spectra were measured on a Mattson 3000 FT-IR spectrometer using a NaCl plate. NMR spectra were recorded with a Jeol Alpha-400 NMR spectrometer (399.65 for ^1H and 100.40 for ^{13}C) using $CDCl_3$ as solvent. MS spectra were recorded on a Jeol AMX505 spectrometer. The HPLC separations were performed using a Beckman M126 pump equipped with a Beckman M168 UV/Vis diode array detector (190-800 nm) detecting at 215 nm.

Microorganism. The fungal strain was isolated from a soil sample containing decayed wood chips, collected at Tikal, Guatemala. Working stocks were prepared on Potato Dextrose agar (22g/l Dehydrated Potato, 20 g/l glucose, 17 g/l agar) slants stored at 4°C. Slants were inoculated from long-term stocks kept at -196°C or from freeze-dried cultures.

Fermentation. Fermentations in the bioreactors were prepared in three different steps; 250 ml flasks containing 30 ml of BGA1 medium (beef extract 0.5%; glycerol 1% and starch 2%; pH 6.5) were seeded from freshly prepared plates and were fermented during 72 hours at 28° C in orbital shakers (250 r.p.m.). 25 ml of these broths were used as inocula for 400 ml fermentations, in BGA1 medium, contained in 2 litre flasks. After 72 hours growth under the above mentioned conditions, 800 ml of the resultant cultures were used to inoculate a 43-litre MBR CH8620 fermenter containing 20 litres of BGA2 medium (beef extract 0.5%; glycerol 1% and

starch 4%; pH 6.5) plus 0.02% SAG 471 Silicon Antifoam (Union Carbide) and 0.18% olive oil. After the sterilization cycle at 121°C for 45 minutes, the medium was cooled to 28°C and inoculated. The fermenters were incubated at 28°C and maintained at 0.5 bar overpressure with an agitator speed of 300 r.p.m. (75 m/min tip speed) and an air flow rate of 10 litres air/min. Set point for pO_2 was adjusted to 80%. Cascade conditions were 750 r.p.m. (maximum) for the 10 litres/min of flow rate.

Extraction and isolation. After 9 days the cultured broth was harvested and centrifuged. 2 litres of the supernatant was extracted with EtOAc (2x2 l) and the combined EtOAc phases were evaporated to dryness to give 1.5 g of a glassy material. This was separated into 25 fractions (70 ml each) on a silica gel column using a step gradient of $CHCl_3$:MeOH (95:5, 600 ml; 90:10, 700 ml; and 80:20, 200 ml) and TLC monitoring (on a silica gel plates with $CHCl_3$:MeOH 9:1 as eluent). Fractions 10 and 11 were combined and purified by reverse phase HPLC with MeCN:H_2O 45:55 on a C_{18} Partisil ODS-2 column (250 x 10 mm i.d) at a flow rate 2.0 ml/minute, affording 0.5 mg of compound **2**, 30 mg of compound **4**, 4.5 mg of compound **5** and 12.0 mg of compound **6** (retention times 37.0, 30.5, 49.8 and 40.9 minutes respectively). Fractions 12 and 13 were combined and purified on the same column as above using MeCN:H_2O 40:60 at a flow rate 2.5 ml/minute affording 7.2 mg of compound **1**, 0.5 mg of compound **3**, 3.0 mg of compound **7**, 1.5 mg of compound **8** and 0.6 mg of compound **9** (retention times 22.7, 28.7, 14.3, 30.4 and 18.0 minutes respectively).

19,20-Epoxycytochalasin R (1): $[\alpha]_D$ -60^0 (c=0.05, $CHCl_3$); IR (NaCl, CCl_4): 3310, 2970,1750, 1690, 1450, 1370, 1230 cm^{-1}; FAB-MS, m/z (rel. int. %): 524 $[M+H]^+$ (100), 91$[C_7H_7]^+$ (30), 43 $[CH_3CO]^+$ (14). ^1H and ^{13}C NMR (see tables 1 and 2).

18-Deoxy-19,20-epoxycytochalasin R (2): IR (NaCl, CCl_4): 2920, 2340,1740, 1690, 1460, 1360 cm^{-1}; FAB-MS, m/z (rel. int. %): 540 $[M+H]^+$ (100), 522 $[M-H_2O]^+$ (10), 91 $[C_7H_7]^+$ (100), 43 $[CH_3CO]^+$ (94). ^1H and ^{13}C NMR (see tables 1 and 2).

18-Deoxy-19,20-epoxycytochalasin Q (5): $[\alpha]_D$ -360^0 (c=0.05, $CHCl_3$); IR (NaCl, CCl_4): 2340, 2300,1750, 1700, 1220 cm^{-1}; FAB-MS, m/z (rel. int. %): 508 $[M+H]^+$ (100), 448 $[M-OAc]^+$ (12), 430 $[M-OAc-H_2O]^+$ (21), 91 $[C_7H_7]^+$ (96), 43 $[CH_3CO]^+$ (94). ^1H and ^{13}C NMR (see tables 1 and 2).

19,20-Epoxycytochalasin N (7): $[\alpha]_D$ -55^0 (c=0.04, $CHCl_3$); IR (NaCl, CCl_4): 3310, 2950, 2300,1740, 1680, 1220 cm^{-1}; FAB-MS, m/z (rel. int. %): 540 $[M+H]^+$ (48), 522 $[M-H_2O]^+$ (10), 480 $[M-CH_3CO]^+$ (25), 91 $[C_7H_7]^+$ (100), 43 $[CH_3CO]^+$ (94). ^1H and ^{13}C NMR (see tables 1 and 2).

19,20-epoxycytochalasin C (8): $[\alpha]_D$ -300^0 (c=0.04, $CHCl_3$); IR (NaCl, CCl_4): 3230, 2930,1750, 1700,1450, 1370, 1230 cm^{-1}; FAB-MS, m/z (rel. int. %): 524 $[M+H]^+$ (45), 463 $[M-CH_3CO]^+$ (12), 91 $[C_7H_7]^+$ (100), 43 $[CH_3CO]^+$ (57). ^1H and ^{13}C NMR (see tables 1 and 2).

19,20-epoxycytochalasin D (9): $[\alpha]_D$ -228^0 (c=0.035, $CHCl_3$); IR (NaCl, CCl_4): 3330, 2930,1750, 1690, 1450,1370, 1220 cm^{-1}; FAB-MS, m/z (rel. int. %): 524 $[M+H]^+$ (43), 480 $[M-CH_3CO]^+$ (18), 463 $[M-OAc]^+$ (15), 91 $[C_7H_7]^+$ (100), 43 $[CH_3CO]^+$ (46). ^1H and ^{13}C NMR (see tables 1 and 2).

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