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Armochaeglobines A and B, Two New Indole-Based Alkaloids from the Arthropod-Derived Fungus *Chaetomium globosum*

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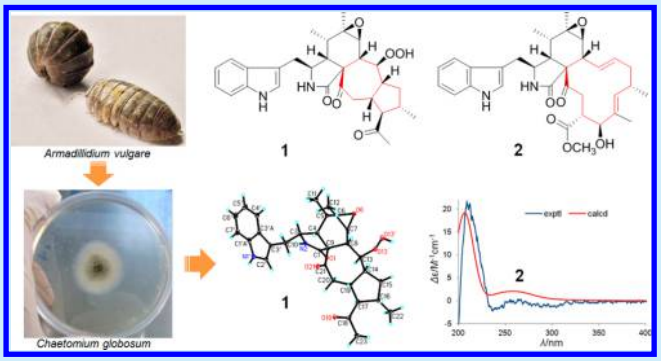
Supporting Information

ABSTRACT: Armochaeglobines A (**1**) and B (**2**), two indole-based cytochalasan alkaloids with new carbon skeletons, were obtained from the fungus *Chaetomium globosum* TW1-1, which was first isolated from the arthropod *Armadillidium vulgare*. Their structures were elucidated by extensive spectroscopic analyses, ECD calculation, and single-crystal X-ray diffraction analysis. Interestingly, compound **1** featured a unique tetracyclic 5/6/7/5 fused ring system and **2** possessed a rare 12-membered carbon scaffold.



Cytochalasans are one class of alkaloids featuring a tricyclic core in which a macrocyclic ring is commonly fused to a perhydroisoindolone ring system.[1] Their complex and highly functionalized structures, associated with the extraordinary range of biological activities, including immunomodulatory,[2] cytotoxic,[3] and nematicidal activities,[4] have attracted great interest from synthetic organic chemistry community as challenging targets for total synthesis.[5] The biosynthetic investigations of cytochalasans have also been a hot topic over the past several years.[6] For example, the molecular basis of cytochalasan biosynthesis in fungi was first demonstrated by Hertweck et al.,[6a,b] and a multifunctional Baeyer−Villiger monooxygenase (CcsB) was reported by Tang et al. to catalyze the formation of an in-line carbonate in the macrocyclic portion of cytochalasin E.[6c] To date, more than 100 natural cytochalasans have been isolated from various fungal sources.[1,3a,7] However, in contrast to considerable efforts toward cytochalasans focused on plant- and marine-derived symbionts,[3a,8] no attention has yet been given to animal-derived fungi, especially those of medicinal animal origins.

In the course of investigating structurally unique and bioactive secondary metabolites from animal-derived fungi, the fungus *Chaetomium globosum* TW1-1, first isolated from the traditional Chinese medicinal arthropods *Armadillidium vulgare*, had been chemically investigated. This work resulted in the isolation of two novel cytochalasans, armochaeglobines A (**1**) and B (**2**), bearing unprecedented 5/6/7/5 tetracyclic and 5/6/12 tricyclic ring systems (Figure 1), together with a new and

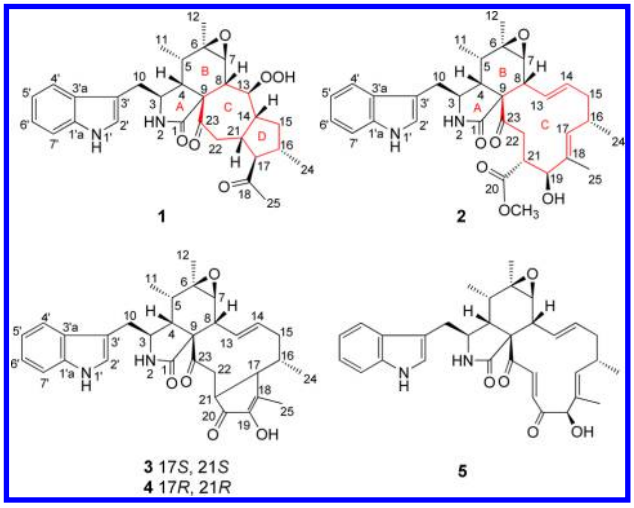


Figure 1. Structures of compounds **1**−**5**.

two known congeners [armochaeglobine C (**3**) and chaetoglobosins U (**4**)[9] and A (**5**)[10]]. It is notable that **1** and **2** represent the first examples of cytochalasans with 10- and 12-membered carbocyclic rings, which differed greatly from those known cytochalasans with 9-, 11-, and 13-membered carbocyclic rings.[1,3] Herein, we present the fermentation, isolation,

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Table 1. ^{1}H NMR Data of Armochaeglobines A−C in CD_3OD (1−3)

no.	1[a]	2[a]	3[b]
3	3.77 ddd (9.0, 5.4, 1.1)	3.72 ddd (8.7, 5.6, 1.1)	3.79 brt (5.7)
4	2.82 dd (6.1, 1.1)	2.63 dd (6.4, 1.1)	2.74 d (6.0)
5	1.66 m	1.67 m	1.69 m
7	3.28 d (6.0)	2.75 d (5.7)	2.90 d (5.9)
8	2.12 dd (9.1, 6.0)	2.23 dd (10.1, 5.7)	2.32 dd (10.4, 5.9)
10a	2.93 dd (14.0, 5.4)	2.87 dd (14.0, 5.6)	2.85 dd (14.2, 4.5)
10b	2.70 dd (14.0, 9.0)	2.42 dd (14.0, 8.7)	2.59 m
11	0.56 d (7.3)	0.63 d (7.3)	0.78 d (7.2)
12	1.18 s	1.14 s	1.18 s
13	4.92 (overlap)	6.16 ddd (15.1, 10.1, 1.5)	6.36 dd (14.9, 10.8)
14	2.56 m (overlap)	5.17 ddd (15.1, 11.2, 3.3)	5.28 brt (13.3)
15α	1.60 ddd (14.8, 9.4, 5.4)	1.82 dt (13.2, 11.5)	2.61 m
15β	2.30 m	2.29 brd (13.2)	1.81 m
16	2.03 m	2.57 m	2.30 m
17	2.37 t (10.2)	4.98 dd (10.5, 1.1)	2.68 m
19	–	4.09 d (10.6)	
21	2.54 m (overlap)	3.37 ddd (10.6, 5.0, 3.4)	2.47 t (6.0)
22a	2.89 m	3.06 dd (20.8, 5.0)	3.09 dd (14.5, 5.3)
22b	2.54 m (overlap)	2.80 dd (20.8, 3.4)	1.82 m
24	1.11 d (6.6)	1.01 d (6.6)	0.63 d (6.8)
25	2.26 s	1.58 d (1.1)	1.88 s
2′	7.10 s	7.03 s	6.96 s
4′	7.54 brd (7.9)	7.45 brd (7.9)	7.48 d (7.8)
5′	7.02 ddd (7.9, 7.1, 0.9)	7.04 ddd (7.9, 7.0, 0.8)	7.07 t (7.3)
6′	7.09 ddd (8.1, 7.1, 1.0)	7.11 ddd (8.1, 7.0, 0.9)	7.01 t (7.3)
7′	7.34 brd (8.1)	7.35 brd (8.1)	7.29 d (8.0)
-OCH3	3.69 s		

[a]600 MHz. [b]400 MHz.

structure elucidation, and bioactivity evaluation, as well as plausible biogenetic pathway, of new skeletons **1** and **2**.

Armochaeglobine A (**1**) was isolated as colorless crystals. Its molecular formula, $C_{30}H_{36}N_2O_6$, with 14 degrees of unsaturation, was deduced from the quasimolecular ion peak at m/z 543.2452 ($[M + Na]^+$) in HRESIMS. The ^{1}H NMR spectrum (Table 1) of **1** showed typical signals assignable to a 3-substituted indolyl and four methyl groups. The ^{13}C NMR (Table 2) and DEPT spectra of **1** displayed resonances for 30 carbons, including three carbonyls (one amide and two ketonic carbonyls), two quaternary carbons, ten methines, three methylenes, four methyls, and another eight signals assignable to the indolyl group. The aforementioned NMR data suggested that **1** was likely a cytochalasan alkaloid.

The complete structure of **1** was determined by correlative analysis of the 2D NMR spectra (Figure S1, Supporting Information, SI) and by comparison of its NMR data with that of chaetoglobosin A (**5**).[10] The isoindolone moiety (rings A and B) of **1** was established to be the same as that of **5** (Figure 1); however, substructures of rings C and D in **1** were quite different from **5** as revealed by 2D NMR spectra. The proton spin system of H-7/H-8/H-13/H-14/H-21/H-22 observed in the ^{1}H−^{1}H COSY spectrum of **1** (Figure S1, SI), along with HMBC correlations from H-4 and H-8 to C-9 and C-23, and from H-21 and H-22 to C-23, established an unusual seven-membered ring C, which was fused to ring B through C-8 and C-9. In addition, a five-membered ring D was elucidated by the ^{1}H−^{1}H COSY spin system of H-14/H-15/H-16/H-17/H-21, and further confirmed by careful inspection of the HMBC spectrum of **1** (Figure S1, SI). Locations of the acetyl group (δ_C 213.9 and 31.5) at C-17 (δ_C 65.6) and the methyl group at C-16 in **1** were determined by HMBC correlations from Me-25 to C-17 and C-18, and from Me-24 to C-15, C-16, and C-17. The presence of the hydroperoxyl group at C-13 was reasonably assigned by the deshielded chemical shift of C-13 (δ_C 83.6) to satisfy the molecular formula required by the HRESIMS.

Table 2. ^{13}C NMR Data of Armochaeglobines A−C in CD_3OD (1−3)

no.	1[a]	2[a]	3[b]	no.	1	2	3
1	176.2	177.0	176.9	18	213.9	134.3	147.3[c]
3	54.6	54.2	54.0	19		81.0	150.2[c]
4	48.0	51.3	49.6	20		176.9	204.7[c]
5	37.5	37.7	38.1	21	43.9	42.6	41.5
6	59.0	58.9	58.9	22	41.2	43.9	43.2
7	61.5	63.3	62.3	23	209.0	208.2	211.3
8	48.1	49.9	51.4	24	20.0	21.3	16.2
9	64.7	66.0	68.3	25	31.5	11.0	12.4
10	33.6	35.2	34.2	1′a	138.1	138.1	138.1
11	12.3	12.6	12.7	2′	124.8	124.5	125.1
12	19.7	19.4	19.5	3′	111.4	110.9	110.4
13	83.6	128.8	131.2	3′a	128.9	128.7	128.8
14	45.6	135.5	133.7	4′	119.2	119.0	119.2
15	39.7	42.7	39.5	5′	119.9	120.0	122.5
16	39.8	33.7	33.0	6′	122.4	122.5	120.1
17	65.6	136.8	44.2	7′	112.4	112.5	112.4

[a]150 MHz. [b]100 MHz. [c]Read from HMBC spectrum; 20-OCH_3 of **2**: 52.5 ppm.

The relative stereochemistry in rings A and B of **1** was established as the same as that of **5** by the analysis of the NOESY spectrum (Figure S1, SI). The NOESY correlation of

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H-7/H-13, coupled with the large coupling constant of H-8/H-13 ($J_{8,13}$ = 9.1 Hz), implied that H-13 should be α-oriented. Furthermore, NOESY correlations from H-14 to H-8 and H-16 suggested that they were cofacial and β-oriented. Since the protons of H-21 and H-22b were overlapped with each other, no useful NOESY interaction could be obtained to clarify the relative configuration of H-21. However, diagnostic NOESY correlations of H-13 with H-17 and H-15α were observed, indicating that **1** contains a *cis*-fused bicyclic rings C and D.

After many attempts, a single crystal of **1** suitable for X-ray analysis was successfully obtained by the vapor-exchange method using methanol and water in a closed tube at room temperature for nearly one month. Through structural refinement, the Hooft parameter is 0.25(8) for 1802 Bijvoet pairs,[11] allowing an explicit assignment of the absolute structure as 3*S*,4*R*,5*S*,6*R*,7*S*,8*R*,9*R*,13*S*,14*R*,16*S*,17*R*,21*R* (CCDC 1025629) (Figure 2). To the best of our knowledge, compound **1** represents the first cytochalasan decorated with a hydroperoxyl group.

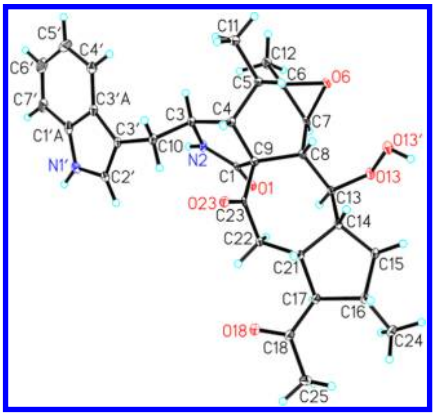

Figure 2. X-ray structure of **1**.

The molecular formula of **2**, $C_{33}H_{40}N_2O_6$, was deduced by HRESIMS. Detailed comparison of the ^{1}H and ^{13}C NMR data of **2** (Tables 1 and 2) with those of **5** also revealed their structural similarities in rings A and B (Figure 1). The main difference between **2** and **5** was observed from C-19 to C-22 in the macrocyclic ring C. Analysis of the ^{1}H−^{1}H COSY and HMBC spectra of **2** (Figure S2, SI) revealed that **2** possessed the same carbon chain from C-13 to C-19 as **5**. However, the ^{1}H−^{1}H COSY correlations of H-19/H-21/H-22 in **2** (Figure S2, SI), along with HMBC interactions from H-21 and H-22 to C-23, and from H-4 and H-8 to C-9 and C-23, constructed a unique 12-membered carbon ring C in **2**, rather than the 13-membered carbon ring in **5** (Figure 1). The methyl ester group at C-21 was verified by the HMBC correlations from H-19, H-21, and the methoxyl protons to C-20. Hitherto, the planar structure of **2** was elucidated.

In the same manner as that of **1**, relative configuration of rings A and B of **2** was also determined to be identical with **1** and **5** by NOESY experiments (Figure S2, SI). The relative configurations of C-16, C-19, and C-21 in the 12-membered ring C of **2** were elucidated by careful analysis of the NOESY spectrum (Figure S2, SI). NOESY correlations from H-14 to H-8 and H-16, and from H-16 to Me-25, indicated that H-8 and H-16 were cofacial and arbitrarily assigned as β-oriented. Consequently, NOESY correlations of H-7/H-13, H-13/H-15α, and H-15α/H-17 suggested their α-orientations. Furthermore, strong NOESY interactions of H-17/H-19 and H-21/Me-25 unambiguously confirmed the α-configuration of H-19 and the β-orientation of H-21. The absolute configuration of **2** was finally determined as 3*S*,4*R*,5*S*,6*R*,7*S*,8*R*,9*R*,16*S*,19*S*,21*R* by theoretical calculation of electronic circular dichroism (ECD, Figure 3).

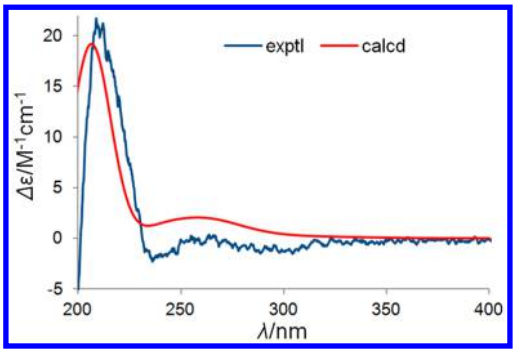

Figure 3. Experimental and calculated ECD spectra of **2**.

The coisolated armochaeglobine C (**3**) was established to be a C-17 and C-21 stereoisomer of **4**.[9] The structure and relative configuration of **3** was elucidated by extensive analysis of 1D and 2D NMR data (Figure S3, SI). Finally, by the same method as for **1**, the crystal of **3** was obtained and the diffraction experiment was carried out. According to the refined Flack parameter value 0.03(17) and Hooft parameter value 0.09(13) for 1939 Bijvoet pairs,[11] the ORTEP drawing for **3** (CCDC 988145) (Figure 4) revealed the absolute configuration as 3*S*,4*R*,5*S*,6*R*,7*S*,8*R*,9*R*,16*S*,17*S*,21*S* with a probability of 1.000.

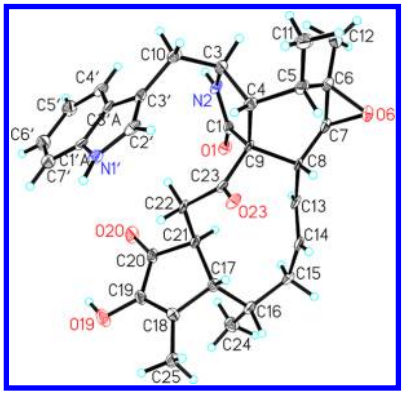

Figure 4. X-ray structure of **3**.

In this study, the biosynthetic pathway of **1** and **2** was outlined in Scheme 1. Both of **1** and **2** might be rationally derived from **5**, which was enzymatically reduced to give penochalasin F (a),[12] and the latter might subsequently involve oxidation, Michael addition, epoxidation, and electrophilic addition, as well as decarboxylation and peroxidation to produce the novel skeleton **1**. In addition, penochalasin F (a) could also be converted to **2** by steps of oxidation, methoxylation, and aldol condensation reactions. The unexpected cycloreversion and rearrangement reactions leading to the complexity of **1** and **2** appeared very distinctive from the biosynthetic pathway proposed for cytochalasans,[6a,b] which might be associated with a series of uncharacterized enzymes of the fungus *C. globosum* TW1-1.

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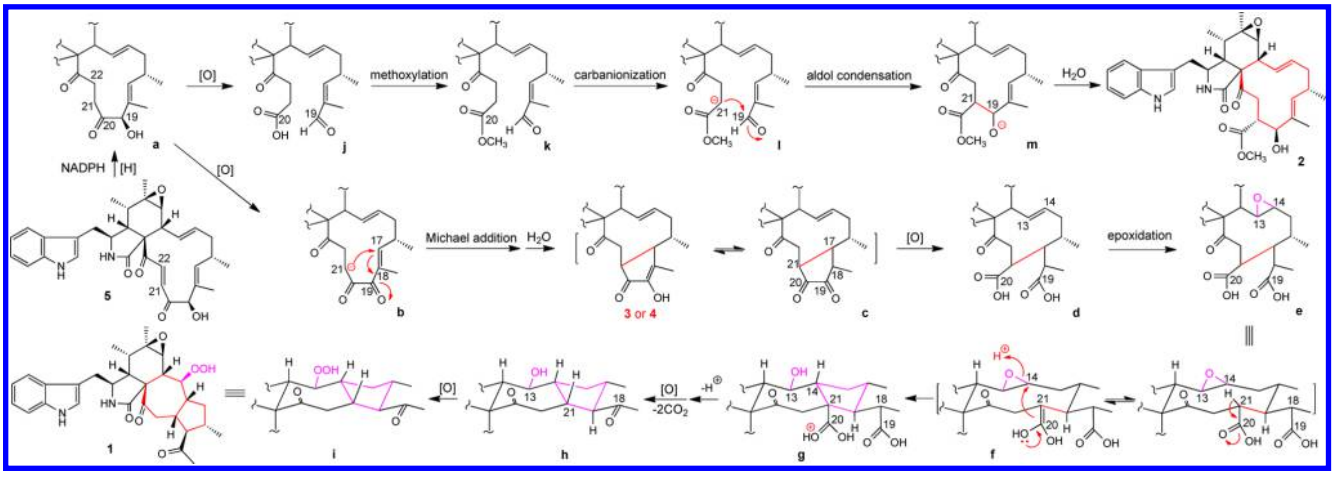
Scheme 1. Plausible Biogenetic Pathway of 1−3



Compounds **1**−**3** were tested for their cytotoxic activities against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480) and the immortalized noncancerous human pulmonary epithelial cell line (Beas-2B) by the MTS method,[13] in vitro, with *cis*-platin as the positive control (Table S1, SI). The results showed that **2** exhibited moderate cytotoxic activities against the tested cancer cell lines with IC_{50} values ranging from 3.4 to 14.7 μM.

Armochaeglobines A (**1**) and B (**2**) represent the first examples of cytochalasans bearing unprecedented 5/6/7/5 tetracyclic and 5/6/12 tricyclic carbon skeletons, which implies **1** and **2** are representatives of two new subclasses of the cytochalasan family with 10- and 12-membered carbocyclic rings, respectively. In particular, **1** possesses an unexpected peroxide functionality, which distinguishes it from all known cytochalasan alkaloids. Further, the decarboxylation and cycloreversion in the proposed biogenetic pathway bring new insight into the biosynthesis of the complex cytochalasans. In summary, the novel carbon frameworks, interesting biological activity, and unique biogenetic pathway of **1** and **2**, together with the noteworthy origin of the fungus *C. globosum* from medicinal arthropod *A. vulgare*, will attract great interest from the pharmacological, synthetic, and biosynthetic communities.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, 1D and 2D NMR, MS, UV, and IR spectra for compounds **1**−**3**, and X-ray crystallographic data of **1** and **3** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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