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Chamiside A, a Cytochalasan with a Tricyclic Core Skeleton from the Endophytic Fungus *Chaetomium nigricolor* F5

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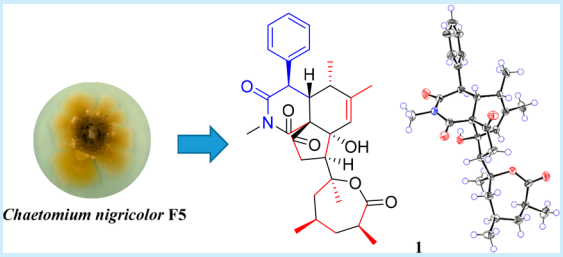
Supporting Information

ABSTRACT: Chamiside A (**1**), a novel cytochalasan with a new 6/6/5-fused tricyclic core skeleton, was isolated from an endophytic fungus, *Chaetomium nigricolor* F5, harbored in the medicinal plant *Mahonia fortunei*. Its structure was unambiguously determined by extensive spectroscopic analyses, measurement of single-crystal X-ray diffraction, and electronic circular dichroism calculation. A biosynthetic pathway for the unique ring system in **1** was proposed. Compound **1** exhibited moderate antibacterial activity against *Staphylococcus aureus*.



Cytochalasans, a large family of fungal secondary metabolites, have attracted considerable attention owing to their intriguing structures and diverse biological activities.[1] Since the discovery of the first cytochalasans in 1966,[2] more than 300 cytochalasans have been discovered from diverse fungal genera, such as *Chaetomium*, *Aspergillus*, and *Periconia* species.[3] The members of cytochalasans are typically characterized by the presence of a substituted isoindole scaffold fused to a macrocyclic ring, derived from a highly reduced polyketide backbone and an amino acid.[4] The variations in size, substitution, rearrangement, and further cyclization of the macrocyclic ring, as well as the incorporation of various amino acids, greatly contribute to the structural diversity and complexity of cytochalasans.[5]

Fungal endophytes asymptomatically colonize healthy plant tissues and have complex association with host plants, parasites, pathogens, and other associated microorganisms.[6] This kind of specific crosstalk enables endophytic fungi to produce skeletally novel and biologically active cytochalasans, such as recently reported antifungal curtachalasins A−E,[7] cytotoxic amichalasines A−C,[8] and periconiasin G with anti-HIV efficacy.[9] During our continuing search for unique bioactive compounds from endophytic fungi inhabiting the medicinal plant *Mahonia fortunei*,[10] an endophytic *Chaetomium nigricolor* F5 was isolated, and LC−HRMS-guided isolation of its secondary metabolites afforded a novel cytochalasan, chamiside A (**1**).

To the best of our knowledge, compound **1** features a novel 6/6/5-fused tricyclic core skeleton bearing a benzene ring and a rare seven-membered lactone. Herein, we report the isolation, structural elucidation, plausible biosynthesis pathway, as well as biological activities of **1**.

Compound **1** (Figure 1) was obtained as colorless crystals. Its molecular formula $C_{30}H_{37}O_6N$ (13 degrees of unsaturation)

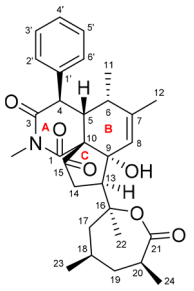


Figure 1. Structure of compound **1**.

was determined from the ESI-HRMS information ($[M + Na]^+$ at m/z 530.2513 and $[2M + Na]^+$ at m/z 1037.5129). The mass spectrum exhibited the loss of one water molecule, revealing the possible presence of one hydroxyl group in **1** (Figure S8). The ^{1}H NMR spectrum (Table 1) suggested the presence of three tertiary methyls (δ_H 1.73, 1.75, and 3.11), three secondary methyls (δ_H 0.44, d, J = 7.5 Hz; δ_H 0.97, d, J = 6.5 Hz; δ_H 1.23, d, J = 7.0 Hz), one olefinic methine (δ_H 5.95), and five aromatic proton signals for a monosubstituted benzene (δ_H 7.22, 2H, d, J = 7.5 Hz; δ_H 7.27, 1H, t, J = 7.5 Hz; δ_H 7.32, 2H, t, J = 7.5 Hz). The 1D NMR data (Table 1) in combination with the HSQC spectrum (Figure S4) confirmed six methyls, three methylenes, six methines, three

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Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of Compound 1 in $CDCl_3$ (δ in ppm)

no.	δ_H (J in Hz)	δ_C	no.	δ_H (J in Hz)	δ_C
1		172.2	16		84.1
2-N			17	1.63−1.71 (2H, overlap)	45.1
3		174.4	18	2.15 (1H, m)	30.0
4	4.02 (1H, d, 12.0)	47.9	19a	1.25 (1H, overlap)	40.8
5	3.19 (1H, dd, 4.0, 12.0)	38.4	19b	1.68 (1H, overlap)	
6	2.28 (1H, m)	33.3	20	2.73 (1H, m)	39.8
7		138.3	21		175.7
8	5.95 (1H, s)	123.9	22	1.75 (3H, s)	24.4
9		78.4	23	0.97 (3H, d, 6.5)	23.7
10		66.2	24	1.23 (3H, d, 7.0)	19.3
11	0.44 (3H, d, 7.5)	16.7	1′		140.8
12	1.73 (3H, s)	21.6	2′, 6′	7.22 (2H, d, 7.5)	128.9
13	3.03 (1H, dd, 8.0, 13.5)	56.4	3′, 5′	7.32 (2H, t, 7.5)	128.9
14a	2.32 (1H, dd, 13.5, 19.0)	40.2	4′	7.27 (1H, t, 7.5)	127.7
14b	2.69 (1H, dd, 8.0, 19.0)		N-CH_3	3.11 (3H, s)	27.5
15		210.9	9-OH	3.47 (1H, s)	

quaternary carbons, eight aromatic/olefinic carbons including a monosubstituted benzene and a double bond, and four carbonyl groups, together with a hydroxyl group (δ_H 3.47, s). These data accounted for all ^{1}H and ^{13}C NMR resonances.

The detailed planar structure of **1** was further constructed by the $^{1}H-^{1}H$ COSY and HMBC data (Figure 2A). Interpretation of the $^{1}H-^{1}H$ COSY spectrum identified the connections from C-4 to C-6, from C-13 to C-14, and from C-17 to C-20. HMBC correlations of H-4/C-3, H-5/C-10, H-5/C-1, N−CH_3/C-1, and N−CH_3/C-3 coupled with the requirement of chemical shifts of C-1 (δ_C 172.2) and C-3 (δ_C 174.4) indicated a piperidine-2,6-dione (A ring, Figure 1) in **1**. The monosubstituted benzene ring was located at the C-4 of ring A, which was supported by the key HMBC correlations of H-4 with C-1′ and C-2′. Based on the HMBC correlations from H-5 to C-9, H_3-11 to C-7, H_3-12 to C-6, C-7, and C-8, as well as 9-OH to C-8, C-9, and C-10, the B ring was deduced and is fused to the A ring. By analyzing the HMBC correlations of 9-OH/C-13, H-13/C-9, H_2-14/C-10, and H_2-14/C-15, the structure of ring C, a cyclopentanone, was elucidated as shown. Furthermore, the fusing pattern of rings A, B, and C is therefore determined. The remaining seven-membered lactone was verified on the basis of the HMBC correlations from H_3-22 to C-16, and C-17, and from H_3-24 to C-21, as well as the chemical shifts of C-16 (δ_C 84.1) and C-21 (δ_C 175.7). The above lactone was determined to be connected to C-13 of ring C by the HMBC correlation of H_3-22 with C-13, which was consistent with the MS and MS/MS requirement (Figure 2B).

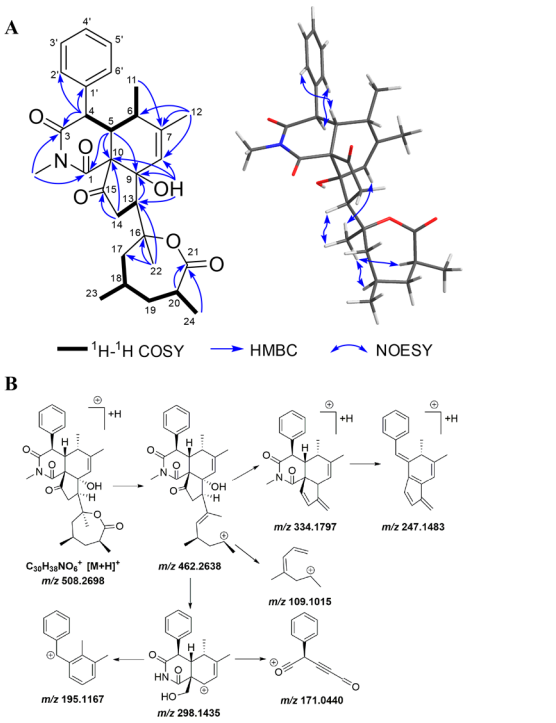

Figure 2. 2D NMR and MS/MS data. (A) Key $^{1}H-^{1}H$ COSY, HMBC, and NOESY correlations. (B) Proposed mass fragmentation pathway.

The relative configuration of **1** was characterized by analysis of the NOESY data and coupling constants (Figure 2A and Table 1). In the NOESY spectrum, the correlations of H_3-22/H-13, H_3-22/H-18, and H_3-22/H-20 were indicative of their being in α-orientations. The large coupling constant of H-4/H-5 (J = 12.0 Hz) and the small coupling constant of H-5/H-6 (J = 4.0 Hz) suggested that H-4, H-5, and H-6 could be α, β, and β configured, respectively. The assignment of the relative configurations of C-9 and C-10 was a big challenge using the NEOSY data because there were no available correlations for 9-OH and the distances between the protons on the rings B and C were far away for NOESY correlation.

After numerous attempts, suitable single crystals for X-ray diffraction were fortunately obtained from ethanol−dichloromethane (5:4). Single-crystal diffraction analysis with Cu Kα radiation confirmed the gross structure and relative configuration and indicated the possible absolute configuration of **1** [Flack parameter was −0.1 (4)] (Figure 3). To further assign the absolute configuration of **1**, ECD calculations were performed at the mPW1PW91/6-311G(d)//B3PW91/TZVP level of theory. The calculated ECD spectrum of **1** is in good agreement with the experimental curve (Figure 4). The absolute configuration of **1** can be determined to be 4*R*,5*S*,6*S*,9*R*,10*R*,13*R*,16*S*,18*R*,20*S*. Therefore, the structure of compound **1** was unambiguously assigned and it was named chamiside A.

Compound **1** features a piperidine-2,6-dione (ring A), which conjugated with a cyclohexene (ring B), and a cyclopentanone

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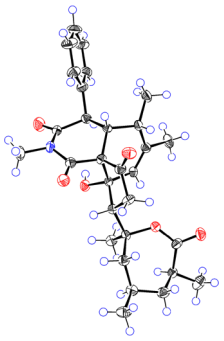


Figure 3. X-ray structure of **1** (ORTEP drawing).

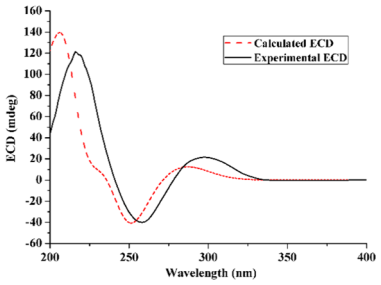
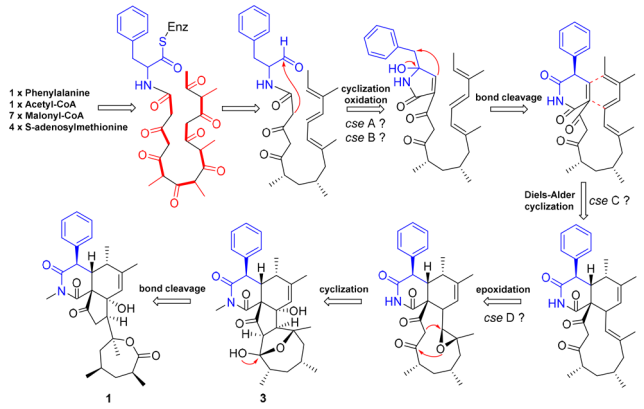


Figure 4. Experimental and calculated ECD of **1**.

(ring C). In addition to these structural characteristics, a rare seven-membered lactone and a benzene ring were linked to the tricyclic core skeleton. Several structural features of **1**, such as ring A, can only be found in previously reported chaetoconvosins A and B (**2** and **3**, Scheme 1 and Supporting Information) with a complex 6/6/5/5/7 pentacyclic ring system,[11] implying that they shared the same biosynthetic intermediate that still remains unclear.

Scheme 1. Proposed Biosynthesis Pathway of 1



Genome sequencing of *C. nigricolor* F5 allowed the identification of a polyketide synthase-nonribosomal peptide synthetase (PKS-NRPS) biosynthetic gene cluster *cse* (Figure S12). It showed high sequence similarity to the cytochalasan chaetoglobosins gene cluster from *Chaetomium globosum* CBS 148.51 (Table S1).

Based on the structure of chamiside A (**1**) and its biosynthetic gene cluster *cse*, the biosynthetic pathway of **1** is proposed as shown in Scheme 1. A linear PKS-NRPS biosynthetic precursor was first constructed from a polyketide chain (octaketide) and an amino acid (phenylalanine) followed by cyclization to drive pyrrolinone formation. Perhaps the most intriguing step in compound **1** biosynthesis is the proposed oxidation and rearrangement in the five-membered pyrrolinone ring to generate the six-membered ring (A ring) (Figures S13 and S14). Further intramolecular [4 + 2] Diels–Alder cycloaddition reaction resulted in the construction of B ring. The subsequent epoxidation, intramolecular nucleophilic addition, rearrangement, or late-stage tailoring of cytochalasan led to the formation of **1**.

Following the established bioassay approaches in our laboratory, chamiside A (**1**) was evaluated for antibacterial activities (against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*), cytotoxic activities (against the human cancer cell line MDA-MB-231), anti-inflammatory activity, and antifungal activity (against *Candida albicans*). Compound **1** showed moderate efficacy against the bacterium *S. aureus* with an MIC of 25 μg/mL.

In summary, chamiside A (**1**) represents a new family of cytochalasans featuring an unprecedented 6/6/5-fused tricyclic core skeleton, further expanding the structural diversity of cytochalasans. Notably, the proposed biosynthetic pathway for the unique core structure of **1** shed new light on the biosynthesis diversity of cytochalasans and is worth unveiling in future research. Therefore, our work will likely form the basis of new explorations into the chemical space of novel chamiside A like cytochalasans.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b01065.

Experimental procedures; 1D and 2D NMR, IR, ESI-HRMS, and MS/MS spectra, together with the biosynthetic gene cluster of compound **1** (PDF)

Accession Codes

CCDC 1904922 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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