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Chaetoconvosins A and B, Alkaloids with New Skeleton from Fungus *Chaetomium convolutum*

Guo-Bo Xu,[†,‡,§] Li-Mei Li,[§,∥] Tao Yang,[†] Guo-Lin Zhang,[*,†] and Guo-You Li[*,†]

Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, P. R. China, Research Center, Chengdu Medical College, Chengdu 610083, P. R. China, and University of Chinese Academy of Sciences, Beijing 100039, P. R. China

zhanggl@cib.ac.cn; ligy@cib.ac.cn

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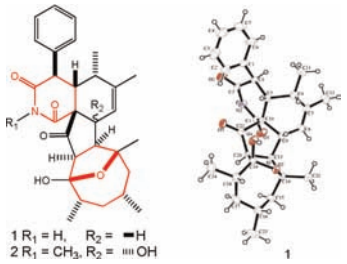
ABSTRACT



Chaetoconvosins A and B (1 and 2), two novel cytochalasan alkaloids with a new 6/6/5/5/7 pentacyclic ring system, were isolated from the solid-state fermented medium of the wheat rhizospheric fungus *Chaetomium convolutum* cib-100. Their structures were elucidated on the basis of spectroscopic data. The structure of chaetoconvosin A (1) was confirmed by X-ray crystallographic analysis. Chaetoconvosin B (2), the major metabolite, showed remarkable inhibitory ability on root elongation and moderate cytotoxicity against several cancer cell lines.

The fungi of the genus *Chaetomium* are widely distributed in air, soil, plant debris, and endophytic habitats.[1] Plenty of metabolites were isolated from about 30 species of this genus. These metabolites possess a broad range of biological activities such as cytotoxic,[2] antimicrobial,[2b,3] antimalarial,[2c,d] antifungal,[4] and plant growth regulation activities.[5] Some fungi of this genus could result in phaeohyphomycosis,[6] onychomycosis,[7] allergy,[8] cutaneous infection,[9] and phytotoxicity.[10]

In the course to study the secondary metabolites of *Chaetomium convolutum*, two novel cytochalasins, chaetoconvosins A and B (**1** and **2**), with a new 6/6/5/5/7 penta ring system were isolated, which featured a piperidine-2,6-dione (ring A), a cyclopentanone (ring C), and a 9-oxabicyclo[4.2.1]nonane with an oxygen bridge between C_{14} and

[†] Chengdu Institute of Biology, Chinese Academy of Sciences
[‡] University of Chinese Academy of Sciences
[∥] Research Center, Chengdu Medical College
[§] These authors contributed equally to this work.

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C_{19} (rings D and E). The piperidine-2,6-dione moiety in compounds **1** and **2** (ring A) was unique among all the cytochalasan-type alkaloids. Moreover, the 9-oxabicyclo-[4.2.1]nonane substructure with three methyls at C-14, C-16, and C-18 was also a novel motif in cytochalasin metabolites. We herein reported the isolation, structural elucidation, and biological activities of the two compounds.

Compound **1** was obtained as colorless crystals with an optical rotation value of $[\alpha]_D^{20}+45°$ (*c* 0.1, CH_3OH). Its molecular formula $C_{29}H_{35}NO_5$ was inferred from the quasi-molecular ion peak at *m*/*z* 500.2381 $[M + Na]^+$ in HRESIMS spectrum, indicative of 13 degrees of unsaturation.

The IR absorptions at 3360, 3210, 1749, 1707, and 1693 cm^{-1} suggested the presence of hydroxyl, amide N−H, and carbonyl groups. The ^1H NMR spectrum of compound **1** revealed characteristic resonances for a monosubstituted phenyl ring [δ_H 7.19 (3H, overlap), 7.28 (2H, overlap)], five methyls [δ_H 0.73 (3H, d, J = 6.5 Hz), 0.78 (3H, d, J = 7.0 Hz), 1.16 (3H, overlap), 1.17 (3H, s), and 1.71 (3H, s)]. The ^{13}C NMR and HSQC experiments displayed the presence of 29 carbon resonances including three carbonyls [one ketonic carbonyl (δ_C 207.5), two amide carbonyls (δ_C 173.4, 172.4)], six phenyl C-atoms (δ_C 140.0, 128.9, 128.9, 128.7, 128.7, 127.1), two olefinic C-atoms (δ_C 139.9, 125.7), three quaternary C-atoms (δ_C 109.5, 79.9, 67.0), eight methines (δ_C 58.2, 54.8, 52.3, 47.6, 45.9, 43.3, 43.3, 37.3), two methylenes (δ_C 52.3, 43.9), and five methyls (δ_C 25.9, 24.4, 19.9, 16.8, 14.5). In view of the unsaturation degree, we could draw a conclusion that compound **1** was a pentacylic cytochalasan-type alkaloid.

The key HMBC correlations of H-4/C-3, C-5, C-6, C-10, C-1′, and C-2′, and H-5/C-1 and C-3 suggested the presence of a piperidine-2,6-dione moiety with a phenyl at the C-4 (ring A). The ring B was postulated by the following HMBC correlations H-4/C-6 and C-10, H-11/C-5, C-6, and C-7, H-12/C-6, C-7 and C-8, and H-5/C-7 and C-21, which was also supported by the similarities of NMR data of ring B in compound **1** with those of chaetoglobsin J,[11] chaetoglobosin

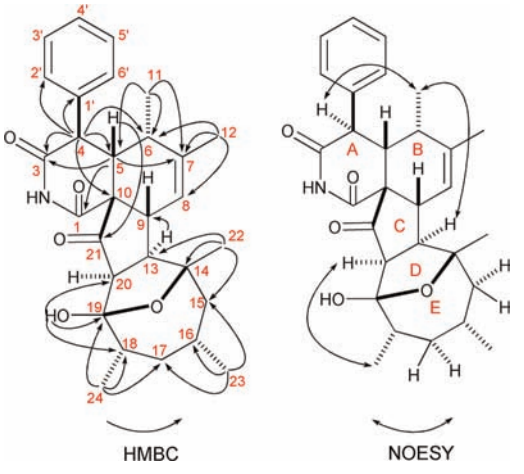

Figure 1. Key HMBC and NOESY correlations of **1**.

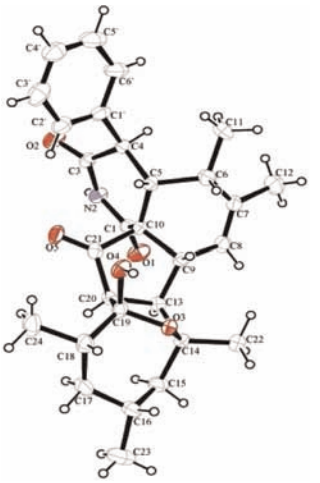

Figure 2. ORTEP diagram of **1**.

T,[2b] and prochaetoglobsin I (Table 1).[12] The moiety of 9-oxabicyclo[4.2.1]nonane (rings D and E) was deduced from the HMBC correlations of H-13/C-9, H-22/C-13, C-14, and C-15, H-23/C-15, C-16, and C-17, H-24/C-17, C-18, and C-19, 19-OH/C-18, C-19, and C-20. The oxygen bridge between C-14 and C-19 could be concluded from the ^{13}C NMR signals at δ_C 109.5 for C-19 (ketal) and 79.9 for oxygenated C-atom (C-14), and the molecular formula. With one unsaturation degree remaining, a carbon−carbon bond should be present between C-13 (δ_C 54.8) and C-20 (δ_C 58.2), which was possibly produced by an intramolecule Michael reaction. The planar structure of compound **1** was thus elucidated as shown in Figure 1. The NOESY correlations of H-11/H-4 and H-13 suggested that H-4, H-11, and H-13 were oriented to the same side. The same orientation of H-20 and H-24 was supported by the NOESY correlation of H-20/H-24 (Figure 1). Unfortunately, other correlations were not observed in the NOESY spectrum. However, the relative configuration of compound **1** was finally determined by X-ray crystallographic analysis (Figure 2).

The molecular formula of compound **2**, white amorphous powder with $[\alpha]_D^{20}+15°$ (*c* 0.1, CH_3OH), was determined to be $C_{30}H_{37}NO_6$ from the quasi-molecular ion peak at *m*/*z* 530.2525 $[M + Na]^+$ (calcd 530.2513) in the HRESIMS, indicating 13 degrees of unsaturation.

The ^1H and ^{13}C NMR spectra of compound **2** shared similarities with those of **1**, indicating that they were analogues. Comparing the ^1H NMR data with those of compound **1**,[13] compound **2** should contain one more methyl (δ_H 2.92, δ_C 27.4) and hydroxyl groups (δ_H 5.77), which were located respectively to N-2 and C-9 in view of the HMBC correlations of CH_3−N with C-1 (δ_C 172.8) and C-3 (δ_C 174.3), and OH with C-9 (δ_C 77.1). The

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Table 1. NMR Data of Chaetoconvosins A and B (**1** and **2**) in DMSO-d_6[a,b]

	1		**2**	
no.	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		172.4		172.8
2-NH	11.13 (1H, s)			
3		173.4		174.3
4	3.90 (1H, d, 2.2)	47.6	4.06 (1H, d, 11.7)	48.2
5	2.79 (1H, dd, 8.5, 2.7)	43.3	2.98 (1H, dd, 11.7, 4.7)	38.1
6	2.69 (1H, m)	37.3	2.25 (1H, m)	32.4
7		139.9		137.8
8	5.85 (1H, s)	125.7	6.02 (1H, s)	123.9
9	2.96 (1H, m)	45.9		77.1
10		67.0		68.0
11	1.16 (3H, overlap)	14.5	0.28 (3H, d, 7.7)	17.1
12	1.71 (3H, s)	19.9	1.54 (3H, s)	22.5
13	3.00 (1H, overlap)	54.8	2.68 (1H, d, 9.1)	63.9
14		79.9		79.5
15a	1.14 (1H, overlap)	52.3	1.07 (1H, dd, 13.7, 11.7)	55.7
15b	1.76 (1H, overlap)		1.68 (1H, overlap)	
16	1.78 (1H, m)	29.1	1.76 (1H, m)	28.3
17a	0.76 (1H, overlap)	43.9	0.74 (1H, overlap)	44.6
17b	1.46 (1H, m)		1.53 (1H, overlap)	
18	1.74 (1H, overlap)	43.3	1.74 (1H, m)	43.1
19		109.5		109.3
20	3.00 (1H, overlap)	58.2	3.06 (1H, d, 9.1)	56.0
21		207.5		207.9
22	1.17 (3H, s)	25.9	1.54 (3H, s)	25.0
23	0.78 (3H, d, 7.0)	24.4	0.79 (3H, d, 6.6)	24.3
24	0.73 (3H, d, 6.5)	16.8	0.83 (3H, d, 6.6)	17.1
1′		140.0		142.4
2′, 6′	7.19 (2H, overlap)	128.7	7.19 (2H, overlap)	128.9
3′, 5′	7.28 (2H, overlap)	128.9	7.28 (2H, overlap)	128.9
4′	7.19 (1H, overlap)	127.1	7.22 (1H, overlap)	127.4
N–CH_3			2.92 (3H, s)	27.4
9-OH			5.77 (1H, s)	
19-OH	5.78 (1H, s)		5.07 (1H, s)	

[a] ^{1}H: 600 MHz; ^{13}C: 150 MHz. [b] Assignments were based on HSQC and HMBC experiments.

structure of compound **2** was finally confirmed by detailed analysis of data of HSQC and HMBC correlations (Figure 3).

The relative configuration of **2** was established by CD spectrum, ROESY, and NOE difference experiments. The Cotton effects in CD spectrum of **2** were similar with that of **1** (see surporting information). The ROESY correlation H-4/H-11 suggested the H-4 and H-11 were at the same side. Correlations of H-20/H-13, H-15$_a$, H-23, and H-24, H-13/H-15$_a$ and H-22 indicated that H-20, H-13, H-15$_a$, H-22, H-23, and H-24 were at the same orientation. The

(13) The chemical shift difference of H-11 between compounds **1** and **2** is possibly derived from the phenyl at C-4. Because of the different orientation of 4-phenyl, H-11 could be in the shielding or deshielding area. In compound **1**, H-11 may be located in the deshielding area of phenyl, but in compound **2**, H-11 may be in the shielding area. The chemical shift value of H-11 is changed in a different solvent at 300 K (δ_H 0.28 ppm in DMSO-d_6, δ_H 0.47 ppm in acetone-d_6 (Supporting Information), which could be from the different conformation of ring A owing to the solvent effect.

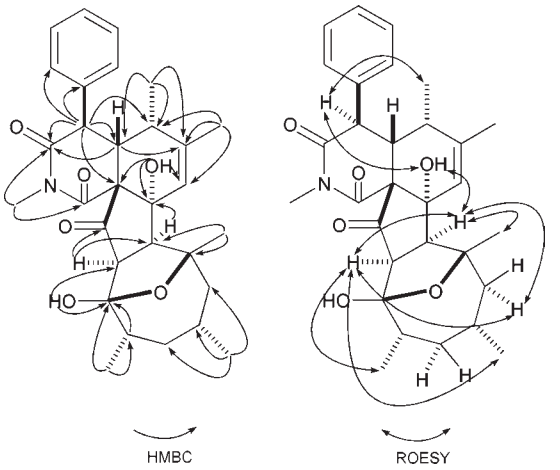
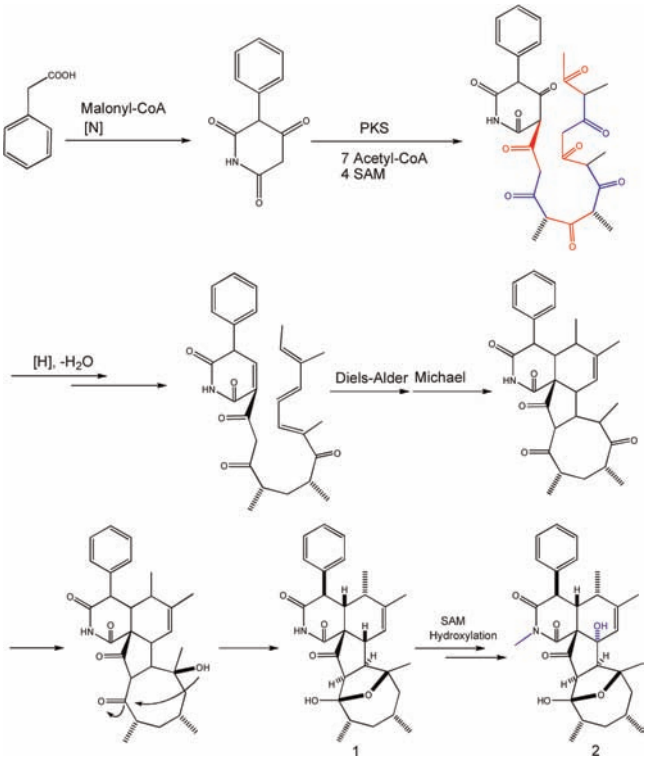


Figure 3. Key HMBC and ROESY correlations of **2**.

relative configuration of 9-OH was determined on the basis of key ROESY correlations of 9-OH with H-4 and H-13 (Figure 3). The same orientation of H-4, 9-OH and H-11 were also confirmed by the NOE difference experiments (see Supporting Information). Therefore, the structure of compound **2** was finally elucidated as shown in Figure 3.

Compound **2** showed moderate cytotoxicity against A549, SMMC-7721, HEPG2, A375, and PC-3 cells with successive IC_{50} values of 26.12, 43.00, 44.60, 47.93, and

Scheme 1. Plausible Biogenetic Pathway of Chaetoconvosins A and B (**1** and **2**)



49.74 μM. Compound **1** exhibited no cytotoxicity against above cell lines ($IC_{50} > 50\ \mu M$) (see Supporting Information Table S1). In seedling assays on wheat (China *L*-30),[14] compound **2** displayed root elongation inhibitory activity. Compound **2** was able to reduce root length by 4.26, 28.73, and 85.24% at respectively concentration of 1.00×10^{-5}, 1.00×10^{-4}, and 4.93×10^{-4} M (see Supporting Information Table S2). Unfortunately, the low available amount of compound **1** precluded the seedling assays.

All of the cytochalasan metabolites, such as cytochalasins, chaetochalasins, and prochaetoglobsins, are possibly biosynthesized via an amino acid and polyketide pathway.[11,15] A possible biosynthetic pathway of chaetoconvosins A (**1**) and B (**2**) is proposed accordingly (Scheme 1). Compounds **1** and **2** represented a novel cytochalasan alkaloid with a new 6/6/5/5/7 penta ring system. Addtionally, chaetoconvosin B (**2**) showed significant root elongation inhibitory activity, indicating that it may be the pathogenic factor to affect wheat growth when *C. convolutum* infects the wheat.

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Supporting Information Available. Experimental section, X-ray crystallographic data of **1**, ^{1}H and ^{13}C NMR, ESIMS, IR, CD, and 2D NMR spectra of chaetoconvosins A and B (**1** and **2**), the NOE difference experiments of **2**, as well as the results for wheat seedling assay and cytotoxicity are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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