Organic LETTERS

Letter

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Aspterpenacids A and B, Two Sesterterpenoids from a Mangrove Endophytic Fungus *Aspergillus terreus* H010

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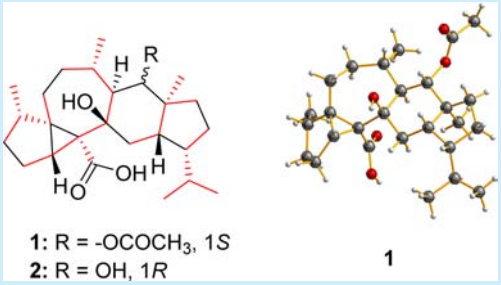
Supporting Information

ABSTRACT: Two new sesterterpenoids, aspterpenacids A (**1**) and B (**2**), with an unusual carbon skeleton of a 5/3/7/6/5 ring system were isolated from the mangrove endophytic fungus *Aspergillus terreus* H010. Their structures were elucidated on the basis of spectroscopic methods, single-crystal X-ray diffraction analysis, and electronic circular dichroism calculations. A biogenetic pathway for **1** and **2** is proposed. Both **1** and **2** showed no significant antibacterial activity or cytotoxicity at 50 μM.



Sesterterpenoids are a relatively small group of terpenoids with widespread sources, which have been found in the metabolites of terrestrial fungi, lichens, plant, insects, and various marine organisms including fungi, sponges, and coral.[1,2] They always possess interesting carbon skeletons including linear,[3] monocyclic,[4] polycyclic,[5,6] and miscellaneous[7] and exhibit diverse biological activities such as antimicrobial,[8] cytotoxicity,[9,10] anti-inflammatory,[11] inhibition of nitric oxide production,[12] and enzyme inhibition.[13,14] In recent years, sesterterpenoids have attracted considerable attention due to their structural conciseness and diverse bioactivity.[1]

In our ongoing search for structurally unique and biologically active metabolites from marine resources,[13−17] a chemical investigation of an endophytic fungus *Aspergillus terreus* H010 was carried out, which was isolated from the mangrove plant *Kandelia obovata*. Two unusual pentacarbocyclic sesterterpenoids, aspterpenacids A (**1**) and B (**2**), with a 5/3/7/6/5 ring system, were obtained (Figure 1). Herein, the details of chemical and biological characterization of the new compounds are reported.

Aspterpenacid A (**1**), which was obtained as a colorless crystal, was deduced to possess the molecular formula $C_{27}H_{42}O_5$ based on the HRESI TOF MS at m/z 445.2956 ([M − H]$^-$, calcd 445.2954), indicating seven degrees of unsaturation. The IR data exhibited absorptions of carboxyl (1679 cm^{-1}), carboxyl ester (1716 cm^{-1}), and hydroxyl (3481 cm^{-1}) functionalities. The ^{1}H NMR spectrum (Table 1) of **1** exhibited signals for four secondary methyls at δ_H 0.81 (d, J = 6.6 Hz, H_3-23), 0.90 (d, J = 6.4 Hz, H_3-24), 0.95 (d, J = 6.7 Hz, H_3-19), 1.11 (d, J = 7.1 Hz, H_3-20); two tertiary methyls at δ_H 0.74 (H_3-25), 2.02 (H_3-2′); and one oxygen-bearing methine at δ_H 4.73 (d, J = 9.6 Hz, H-1).

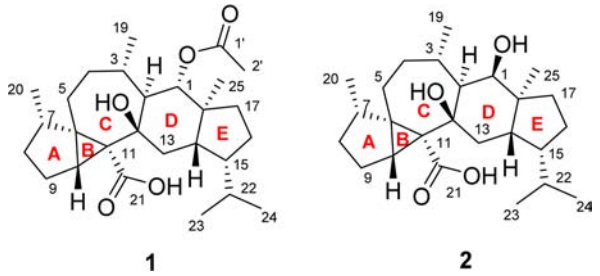


Figure 1. Structures of **1** and **2**.

The ^{13}C NMR and DEPT spectra (Table 1) revealed 27 carbon resonances composed of two carbonyl carbons and 25 aliphatic carbons including six methyls, eight methylenes, seven methines, and four quaternary carbons. A comprehensive analysis of 1D NMR data and degrees of unsaturation revealed that **1** should be an acetylated sesterterpenoid with a pentacyclic structure. Further analysis of the 2D NMR experiment was carried out to establish the planar structure of **1**. The ^{1}H−^{1}H COSY spectrum (Figure 2) indicated the presence of three independent spin systems of H-1/H-2/H-3(H-3/H_3-19)/H_2-4/H_2-5, H_3-20/H-7/H_2-8/H_2-9/H-10, and H_2-13/H-14/H-15(H-15/H-22/H_3-23, H-22/H_3-24)/H_2-16/H_2-17. The HMBC (Figure 2) showed the correlations from the methyl group (H_3-25) to C-1, C-14, C-17, and C-18, from H-1, H-2, H_2-13, and H-14 to C-12, which established the ring D and E systems. The structure of ring C was deduced by the HMBC correlations (Figure 2) from H_2-13 and

Received: February 2, 2016
Published: March 3, 2016

DOI: 10.1021/acs.orglett.6b00336
Org. Lett. 2016, 18, 1406−1409

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data of 1 and 2

	1[a]		2[b]	
position	δ_H (mult, *J*, Hz)	δ_C	δ_H (mult, *J*, Hz)	δ_C
1	4.73 (d, 9.6)	83.7 CH	3.68 (d, 2.5)	74.9 CH
2	2.46 (t, 9.6)	48.1 CH	2.10 (m)	45.6 CH
3	1.80 (m)	36.3 CH	1.96 (m)	33.3 CH
4	1.68 (m)	37.9 CH_2	1.73 (m)	36.0 CH_2
	1.74 (m)		1.78 (m)	
5	1.86 (m)	32.4 CH_2	1.76 (m)	31.6 CH_2
	2.10 (m)		2.15 (m)	
6		42.4 C		42.0 C
7	2.18 (m)	46.4 CH	2.19 (m)	46.1 CH
8	0.90 (m)	31.6 CH_2	1.02 (m)	31.1 CH_2
	1.65 (m)		1.62 (m)	
9	1.94 (m)	26.9 CH_2	1.90 (m)	26.1 CH_2
	2.11 (m)		2.09 (m)	
10	1.65 (m)	32.0 CH	1.70 (m)	31.0 CH
11		43.5 C		41.9 C
12		77.0 C		76.9 C
13	1.77 (m)	39.3 CH_2	1.93 (m)	38.8 CH_2
	2.15 (m)		1.99 (m)	
14	1.92 (m)	41.7 CH	2.13 (m)	35.6 CH
15	1.81 (m)	46.4 CH	1.72 (m)	46.1 CH
16	1.38 (m)	27.8 CH_2	1.55 (m)	27.2 CH_2
	1.77 (m)		1.84 (m)	
17	1.37 (m)	40.1 CH_2	1.15 (m)	33.2 CH_2
18		47.7 C	1.71 (m)	45.3 C
19	0.95 (d, 6.7)	23.2 CH_3	1.02 (d, 6.4)	21.5 CH_3
20	1.11 (d, 7.1)	14.1 CH_3	1.12 (d, 7.0)	13.8 CH_3
21		179.9 C		175.5 C
22	1.57 (m)	31.1 CH	1.55 (m)	31.0 CH
23	0.81 (d, 6.6)	21.9 CH_3	0.84 (d, 6.5)	21.8 CH_3
24	0.90 (d, 6.4)	24.1 CH_3	0.94 (d, 6.3)	23.3 CH_3
25	0.74 (s)	14.1 CH_3	0.74 (s)	17.2 CH_3
1′		171.2 C		
2′	2.02 (s)	21.9 CH_3		

[a]Measured in chloroform-*d*. [b]Measured in a mixture of chloroform-*d* and methanol-d_4.

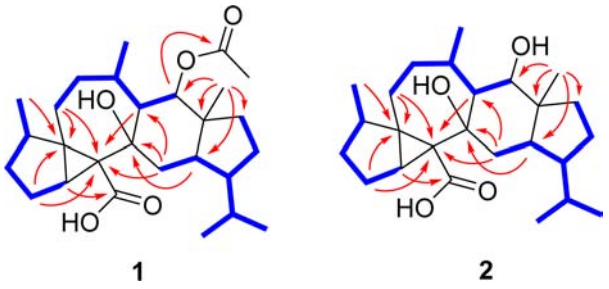


Figure 2. ^{1}H−^{1}H COSY (blue lines) and key HMBC (red arrows) correlations of **1** and **2**.

H-2 to C-11 and H_2-5 to C-6 and C-11. Furthermore, the correlations from H_3-20 and H_2-9 to C-6 were detected, which constructed a spirane structure composed by ring A and ring C. The presence of the three-membered ring (ring B) was supported by the HMBC correlations from H_2-9 to C-11 and an additional correlation from H-10 to the carbonyl carbon (C-21) located at the carboxyl group (C-21) at C-11. Finally, the chemical shift of C-12 (δ_C 77.0) indicated that a hydroxyl group was attached, and the acetyl group should be linked to C-1 based on the correlation from H-1 to C-1′ in the HMBC spectrum. Hence, the planar structure of aspterpenacid A (**1**) was elucidated as shown.

The relative configuration of **1** was determined by analysis of the NOESY data (Figure 3). The cross-peaks of H-2/H_3-19/H_3-2′/H_3-25, H_3-25//H-22, and H_3-20/H_3-19 revealed that they were cofacial and were assigned to be α-oriented. The β-direction of H-14 was supported by the correlations of H-14/H-1. To assign the stereochemistry of the hydroxyl group at C-12, the NOESY experiment was recorded again in DMSO. The correlation of 12-OH/H-14/H-1 revealed the 12-OH was also β-oriented. Meanwhile, a further NOE interaction of H-10/OH-12/H-14 in DMSO indicated the β-orientation of the three-membered ring and H-10. The relative configuration of **1** was further comfirmed by a single-crystal X-ray diffraction experiment using Cu Kα radiation (Figure 4).

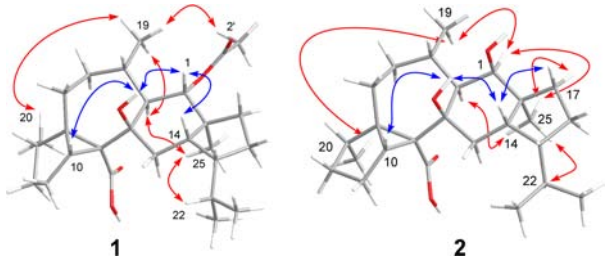


Figure 3. Key NOESY correlations of **1** and **2**.

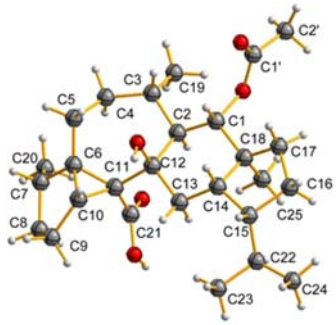


Figure 4. X-ray crystallographic analysis of **1**.

To establish the absolute configuration of **1**, the electronic circular dichroism (ECD) spectrum of **1** was recorded in MeOH and compared with the DFT-calculated spectra of two feasible configurations 1*S*,2*S*,3*S*,6*S*,7*S*,10*R*,11*R*,12*R*,14*S*,15*R*,18*S* and 1*R*,2*R*,3*R*,6*R*,7*R*,10*S*,11*S*,12*S*,14*R*,15*S*,18*R* (**1** and *ent*-**1**, respectively) at the RB3LYP/6-311+G(2d,p) level. The calculated ECD spectrum of **1** showed an excellent fit with the experimental plot (Figure 5), which supported the absolute configuration being 1*S*,2*S*,3*S*,6*S*,7*S*,10*R*,11*R*,12*R*,14*S*,15*R*,18*S*. Thus, the completed structure of **1** was elucidated as depicted in Figure 1.

Aspterpenacid B (**2**) was isolated as colorless powder and assigned a molecular formula of $C_{25}H_{40}O_4$ based on the HRESI TOF MS at *m*/*z* 403.2848 ([M − H]$^-$, calcd 403.2854). The IR absorption (1682 cm^{-1}) suggested that a carboxyl group was observed in **2**. Comparison of the 1D NMR spectra of **2** with those of **1** (Table 1) revealed that they shared the same carbon skeleton of sesterterpenoid. Nevertheless, the clearest differences observed were the absence of signals of the methyl (δ_H 2.02) in the ^{1}H NMR and two carbons, including a carbonyl carbon (δ_C

DOI: 10.1021/acs.orglett.6b00336
Org. Lett. 2016, 18, 1406−1409

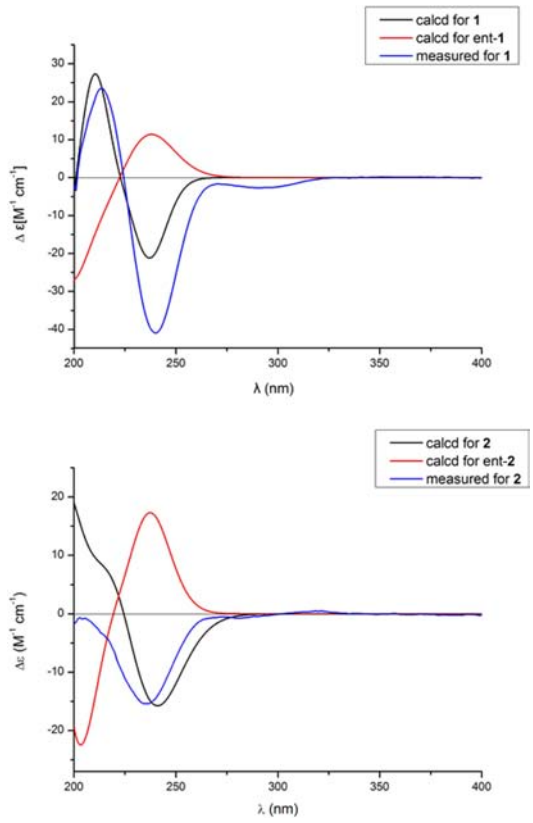


Figure 5. Comparison of the measured ECD spectra of **1** and **2** with the RB3LYP/6-311+G(2d,p) calculated spectrum of their enantiomers in methanol.

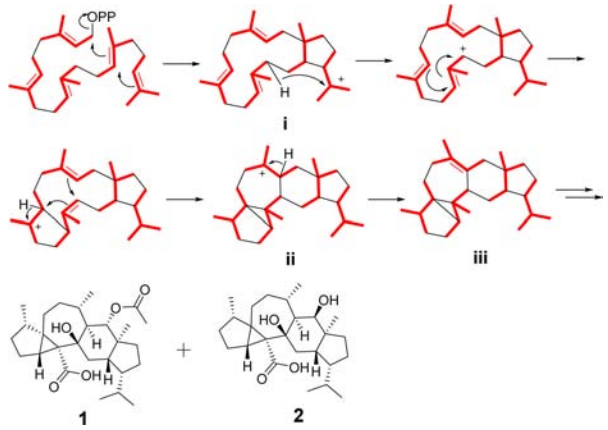
171.2) in the ^{13}C NMR, which indicated that compound **2** was the deacetylated product of **1**. The gross structure of **2** was further confirmed by the analysis of 2D NMR (Figure 2), as shown in Figure 1.

The stereochemistry of **2** is deduced by the NOESY measured in CD_3OD (Figure 3). The α-orientation of H-2, H_3-19, H_3-20, H-22, and H_3-25 was elucidated based on the NOE interactions of H-2/H_3-19/H_3-25, H_3-25/H-22, and H_3-19/H_3-20, and the β-direction of H-14 was supported by the correlations of H-14/H-17β and H_3-25/H-17α. However, the correlation between H-1 and H-14 was absent, and an additional NOE interaction of H-1/H_3-19 was observed, indicating that H-1 was the α-direction, which was reversed compared with **1**. Furthermore, the NOESY spectrum measured in DMSO gave the correlation of 12-OH/H-14/1-OH, revealing that the hydroxyl group at C-12 (12-OH) was β-oriented. Finally, the orientation of the three-membered ring and H-10 was the same as that in **1** and assigned as β-oriented, which was confirmed by a NOE interaction of H-10/OH-12/H-14. All of these facts showed that the stereochemistry of C-1 was different compared with that in **1**, and this was further confirmed by the coupling constant value of $J_{1ax/2ax}$ = 9.6 Hz in **1** and $J_{1eq/2ax}$ = 2.5 Hz in **2** (Table 1).

The absolute configuration of **2** was determined by the comparison of the ECD spectra recorded in MeOH and the DFT-calculated spectra of two feasible configurations, 1*R*,2*S*,3*S*,6*S*,7*S*,10*R*,11*R*,12*R*,14*S*,15*R*,18*S* and 1*S*,2*R*,3*R*,6*R*,7*R*,10*S*,11*S*,12*S*,14*R*,15*S*,18*R* (**2** and *ent*-**2**, respectively), at the RB3LYP/6-311+G(2d,p) level. The calculated ECD spectrum of **1** showed an excellent fit with the experimental spectrum (Figure 5), which indicated the absolute configuration to be 1*R*,2*S*,3*S*,6*S*,7*S*,10*R*,11*R*,12*R*,14*S*,15*R*,18*S*. Thus, the completed structure of **2** was established as shown in Figure 1.

To the best of our knowledge, aspterpenacids A (**1**) and B (**2**) represent a noval carbocyclic skeleton containing an unprecedented 5/3/7/6/5 ring system. The hypothetical biosynthetic pathway for **1** and **2** is proposed in Scheme 1, which is derived from geranylfarnesyl pyrophosphate (GFPP) and followed by a series of cyclizations, rearrangements, and redox reactions. The initial head-to-tail connection of GFPP and cyclization by removing the pyrophosphate moiety give the intermediate a 15-membered ring (structure i). The following formation from the 15/5 ring system to the 5/6/7/3/5 carbon skeleton (structure i) is accompanied by hydrogen migration and carbon cyclization. Finally, a further oxidation, reduction, and acetylation of intermediate iii can generate **1** and **2**.[13,14]

Scheme 1. Plausible Biogenetic Pathway of 1 and 2



Aspterpenacids A (**1**) and B (**2**) were assayed for their antibacterial effect against three Gram-positive and three Gram-negative strains (see the Supporting Information). However, neither exhibited significant activity at 50 μM. The cytotoxicity of **1** and **2** was also tested against HeLa and MCF-7 cancer cell lines using the MTT method, but no activity was detected.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00336.

Experimental section, NMR spectra, HRESI TOF MS, and computational details of **1** and **2** (PDF)
X-ray crystallographic data for **1** (CIF)

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Author Contributions

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the National Natural Science Foundation of China (21472251, 41276146, 41404134), the Science & Technology

DOI: 10.1021/acs.orglett.6b00336
Org. Lett. 2016, 18, 1406−1409

Plan Project of Guangdong Province of China (2013B021100011), Special Financial Fund of Innovative Development of Marine Economic Demonstration Project (GD2012-D01-001), China's Marine Commonweal Research Project (201305017), and the Fundamental Research Funds for the Central Universities (14lgjc16) for generous support.

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DOI: 10.1021/acs.orglett.6b00336
Org. Lett. 2016, 18, 1406−1409