NOTE

Aspochalasin U, a moderate TNF- α inhibitor from *Aspergillus* sp.

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The Journal of Antibiotics (2012) 65, 49–52; doi:10.1038/ja.2011.97; published online 26 October 2011**Keywords:** *Aspergillus*; aspochalasin U; cytochalasin; TNF- α

Tumor necrosis factor- α (TNF- α) is a pleiotropic cytokine that mediates biological activities in many immune-mediated inflammatory diseases such as rheumatoid arthritis, psoriasis, septic shock and inflammatory bowel disease. Blockage of the effect of TNF- α has been proved efficient for treating these diseases.¹ Three TNF- α antagonists, infliximab, adalimumab and etanercept, the former two being monoclonal antibodies and the latter a soluble receptor, have been licensed for clinical use for the treatment of certain immune-mediated inflammatory diseases since 1998, with the mechanism of neutralizing the excess TNF- α at inflammatory sites.² Although these protein-based therapeutics have shown remarkable efficacy, more and more reported adverse responses in patients, and about only 50% or fewer rheumatoid arthritis patients achieved a 50% response in most clinical trials.² Side effects, combined with high treatment payments, spur the development of new therapeutic agents for immune-mediated inflammatory diseases. Many natural compounds have been found to have the capability of reducing TNF- α levels, so small-molecule natural products with the advantage of a convenient route of administration and the facility of maintaining the production of compounds hold significant promise for a new cost-effective alternative to protein-based therapeutics.

The marine environment has been described as a promising source of novel nature product with chemical diversity, for many bioactive compounds have been isolating from marine fungi and actinobacteria. Moreover, many compounds that originally regarded as the productions of marine higher organisms, were later proved to be the products of host-associated microorganisms.³ During the past 10 years, our group has been engaged in the isolation of microorganisms from marine sources, including mangroves, sea-bed mold and salterns. In an effort to find new TNF- α inhibitors, a library containing >7000 isolates has been established. One strain *Aspergillus* sp., F00685 (collected in China Center for Type Culture Collection, Wuhan, Hubei Province, China, no.: M2011179), isolated from the Dongshi Saltern, produced a new cytochalasin, aspochalasin U (1), with six known cytochalasin-type compounds. Compound 1 exhibited mod-

erate anti-TNF- α activity in L929 cell line. This paper describes the isolation and structure elucidation of compound 1.

The strain F00685, isolated from Dongshi Saltern, Fujian, China, was tentatively grouped with the genus *Aspergillus* sp. based on their colony morphological feature. Although it was purified by potato-dextrose-agar medium with 6% (w/v) NaCl, it cultured well with different NaCl concentrations (0~9%) (w/v), which suggested this strain was not an obligate halophile.

The strain was cultured on potato-dextrose-agar medium (30l), which consisted of potato 200 g (diced, boiled for 30 min and filtered, kept the filtrate), dextrose 20 g and agar 15 g in 1 l of seawater at 28 °C for 14 days. The mycelial cake was immersed in EtOAc–MeOH–AcOH (80:15:5, in volume) to extract the metabolites for three times. The crude extract (18 g) was fractionated by reverse-phase C₁₈ (170 g) medium-pressure liquid chromatography (H₂O–MeOH, 0:100, 30:70, 50:50, 70:30, 100:0, in volume, 200 ml each proportion, flow rate of 20 ml min⁻¹). The 50:50 eluates were collected for further chromatography on Sephadex LH-20 (140 g, Qingdao Haiyang Chemical Co., Ltd, Qingdao, Shandong Province, China) in MeOH, the fractions including 1 were combined for another Sephadex LH-20 (40 g) chromatography in acetone (Me₂CO) to yield 1 (10 mg), with *R*_f value of 0.5 on GF₂₅₄ thin-layer chromatography plate (CHCl₃–MeOH, 10:1, v/v).

Compound 1 (Figure 1) was obtained as colorless needle crystal (m.p., 208–210 °C). Its molecular formula was determined to be C₂₄H₃₇NO₅ based on high-resolution ESI mass spectrum (HRESIMS) data (Supplementary Information S14), which showed pseudomolecular ions at *m/z* 442.2566 [M+Na]⁺ (calculated: 442.2569) with seven unsaturations. ¹H and ¹³C NMR spectra in combination with DEPT and ¹H–¹³C HSQC spectra (Supplementary Information S1–S7; Table 1) revealed the presence of five methyl groups, five multiplet methylene groups, eight methine groups (including one sp² methine (C-13: δ _H 5.97, δ _C 123.6), three oxygen-substituted methines (C-7: δ _H 3.96, δ _C 69.7; C-18: δ _H 3.45, δ _C 73.1 and C-19: δ _H 3.61, δ _C 68.2)), six quaternary carbons (including three olefinic carbons (C-5: δ 126.7, C-6: δ 133.4 and C-14: δ 138.7) and two carbonyl carbons (C-1: δ 175.8 and C-21: δ

209.2)), consistent with the molecular formula. As four out of seven unsaturations were accounted for, it was deduced that **1** had three rings.

Inspection of ^1H - ^1H COSY and HMBC spectra (Supplementary Information S8 to S11) allowed for the deduction of the gross structure of **1** as shown in Figure 2. Based on ^1H - ^1H COSY, the spin systems beginning with 23-, 24- CH_3 and continuing through to 4-CH, as well as the fragments from 7-CH to 13-CH and from 15- CH_2 to 20- CH_2 , could be elucidated. The key HMBC correlations from H-4 to C-5, C-6, C-9 and C-1, from H-7 to C-5 and C-6, from H-8 to C-4, C-6, C-9 and C-1, as well as the cross peaks from 11- CH_3 and 12- CH_3

to C-3, C-4, C-5, C-6 and C-7, established the skeleton of dimethyl substituted sperhydroisindol-1-one.⁴ Taking account of only one unsaturation left, along with the fragment from C-15 to C-20 obtained from ^1H - ^1H COSY correlations, as well as the HMBC correlations from H-25 to C-8, C-13, C-15, C-16 and from H-4 to C-21 ($\delta_{\text{C}}=209.2$, a typical ketone carbonyl shift), a nonanoyl substructure fused with the perhydroisindol-1-one at C-8 and C-9 was deduced. The structure skeleton demonstrated that it belonged to a leucine-derived cytochalasin called aspochalasins. The unique positions of three hydroxyls lead it to a new aspochalasin, named aspochalasin U, analogous to the previously known aspochalasin L.⁵

The relative stereochemistry of **1** was determined with comprehensive spectral analysis of NOESY (Supplementary Information S12; Figure 3). The correlations between H-4 and H-8 were observed, which confirmed the fact that in all cytochalasins isolated so far, the 5/6 ring junction and the macrocyclic ring are *cis*- and *trans*- stereochemistry, respectively. It is reported that this is the absolute configuration of cytochalasins because of the diastereofacial selectivity of the cycloaddition reaction during the biosynthesis, which assigned the absolute configurations for C-3, C-4, C-8 and C-9 as 3*S*, 4*R*, 8*R* and 9*R*, respectively.^{6,7} The cross peaks between H-8, CH_3 -25 and Hb-15, and between H-13 and Ha-15 established the *E* configuration for the C-13(14) bond on the macrocyclic ring. The correlations between H-13 and H-7, and between H-13 and H-18 led to the determination of the stereochemistry of C-7 and C-18. The fact that H-18 and H-19 correlated with Hb-20 and Ha-20, respectively, indicated that C₁₉-OH has an orientation opposite to that of C₁₈-OH. We further confirmed

Figure 1 Structure of aspochalasin U (**1**).

Table 1 NMR spectral data for aspochalasin U (**1**) in CD₃OD

Position	δ_{C}	δ_{H} (mult. J in Hz)	COSY	HMBC	NOESY
1	175.8 C				
2					
3	55.1 CH	3.35 (m)	H-4, H-10	1, 5, 9, 10, 22	
4	51.3 CH	3.07 (br s)		1, 3, 5, 6, 9, 10, 11, 21	H-8
5	126.7 C				
6	133.4 C				
7	69.7 CH	3.96 (d, 10.0)	H-8	5, 6, 13	H-13
8	47.5 CH	2.62 (t, 10.0)	H-7, H-13	1, 4, 6, 7, 9, 13, 14, 21	H-4
9	62.2 C				
10	45.3 CH_2	1.21 (m, 6.6)	H-3, H-22	3, 4, 22, 23, 24	H-4
11	16.5 CH_3	1.77 (s)		4, 5, 6, 7	H-3, H-4
12	13.4 CH_3	1.74 (s)		3, 4, 5, 6, 7	H-7
13	123.6 CH	5.97 (d, 10.0)	H-8	15, 25	H-15a
14	138.7 C				
15	39.7 CH_2	2.01 (br t, 11.0) 2.16 (m, 11.0)	H-16a H-16, H-19	13, 14, 16, 17, 25	H-13
16	18.6 CH_2	1.54 (m) 1.62 (m)	H-15a H-15b, H-17b	13, 14, 15, 17	
17	29.5 CH_2	1.44 (dd, 3.0, 15.0) 1.86 (m)	H-18 H-16b, H-18	16	
18	73.1 CH	3.45 (m, 4.9)	H-17, H-19	16, 19, 20	H-13, H-20b
19	68.2 CH	3.61 (m, 4.9)	H-18, H-20	17, 18, 21	H-20a
20	45.7 CH_2	2.19 (m, 6.0) 3.77 (dd, 18.0)		18, 19, 21	H-17a
21	209.2 C				
22	24.8 CH	1.63 (m, 6.6)	H-10	3, 10, 23, 24	
23	21.1 CH_3	0.93 (d, 6.6)	H-22	10, 22, 24	
24	21.9 CH_3	0.95 (d, 6.6)	H-22	10, 22, 23	
25	14.6 CH_3	1.49 (s)		7, 8, 9, 13, 14, 15, 16	H-8, H-15b

Spectra were recorded on Bruker DRX 600 MHz NMR spectrometers (Bruker, Zug, Switzerland) using TMS as internal standard.

Figure 2 H-H COSY and key HMBC correlations of **1**. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

Figure 3 NOESY of **1**.

Figure 4 The final X-ray structure of **1**.

the configurations with the X-ray diffraction structure of compound **1** (Figure 4, the X-ray diffraction data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif (CCDC-833246). The absolute stereochemistry *S* of C7 was also established by a modified Mosher ester method (Figure 5). Thus, the complete absolute configurations of **1** were assigned as (3*S*, 4*R*, 7*S*, 8*R*, 9*R*, 13*E*, 18*R* and 19*R*).

Figure 5 $\Delta\delta$ values (in p.p.m.) obtained from (S)- and (R)-MPTA esters **1a** and **1b**.

Figure 6 Dose-dependent action of **1**.

Anti-TNF- α activity of **1** against mouse fibroblast cell line L929 was tested with TNF- α at 3 ng ml⁻¹ for 24 h by WST-8 colorimetric assay (Cell Counting Kit, Dojindo, Japan). The TNF- α -inhibitory activity of **1** exhibited dose-dependent manner (Figure 6), the survival rate of L929 cell lines rose from about 30% to 53% when the concentration of **1** changed from zero to 75 mg ml⁻¹ ($EC_{50} > 100$ mg ml⁻¹), which indicated that **1** had moderate activity against the necrotic cell death induced by TNF- α . This is the first report that cytochalasan-type compounds exhibit TNF- α inhibitory activity, while the detailed biological activity and identified target of **1** are on the way to elucidate.

Many natural products, such as phenolics, terpenes and alkaloids, have been found to inhibit the upstream signaling pathways to inhibit the expression of TNF- α ,⁸ but there is no lead compound that can inhibit the excessive TNF- α or its downstream pathways. Here, we reported a new cytochalasan, aspochalasin U, that was prepared from the strain *Aspergillus* sp. F00685, isolated from the Dongshi Saltern and exhibited moderate anti-TNF- α activity, which inhibited the excessive TNF- α . This result should encourage the discovery of an alternative approach for the treatment of immune-mediated inflammatory diseases by modulation of the TNF- α signaling pathway.

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