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NOTE

New aliphatic acid amides from *Streptomyces maoxianensis* sp. nov.

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Unsaturated fatty acid amides, which formed from an unsaturated fatty acid and an amine, have diverse biological potency including the modulatory effect on isolated gastrointestinal tract, the effect on transactivational activity of peroxisome proliferator-activated receptors (PPARs) and the cholesterol acyltransferase inhibitory activity.[1–3] At present, more than 20 unsaturated fatty acid amides have been isolated from *Zanthoxylum piperitum*, such as α-sanshool, β-sanshool, hydroxyl-γ-sanshool and ZP-amide A–F, which possessed unsaturated aliphatic acids conjugated with isobutylamine or its derivatives.[2–6] To our knowledge, natural compounds possessing a 12-carbon polyketide conjugated with L-isoleucine (or derivatives), for example, curvularides A–E and coronatine, have been obtained from microbial resources.[7,8] In the course of hunting for new biologically active compounds from microbial sources, two new unsaturated fatty acid amides, maoxianamides A (**1**) and B (**2**), were isolated from *Streptomyces maoxianensis* sp. nov. Herein, we report details of the isolation, structure elucidation and biological activity of the two new compounds.

The producing strain *S. maoxianensis* sp. nov. was isolated from a soil sample collected from a pine forest in Songpinggou, Maoxian, southwest China, which has been reported in the previous paper.[9] The GenBank/EMBL/DDBJ database accession number of the 16S ribosomal RNA sequence of strain is KF887908 and it was deposited in the China General Microbiological Culture Collection Center (CGMCC) with accession CGMCC No. 4.7139.

This strain was grown and maintained on the medium containing 4 g yeast extract, 4 g glucose, 10 g malt extract and 20 g agar in 1.0 l tap water, pH 7.0–7.2 and incubated for 6–7 days at 28 °C. The strain of stock culture was transferred into 1 l Erlenmeyer flasks containing 25% volume of the seed medium and incubated at 28 °C for 24 h, shaken at 150 r.p.m. Then, 1 l of the culture was transferred into a 50 l fermentor containing 30 l of producing medium consisting of 10 g glucose, 40 g soluble amylum, 5 g yeast extract, 25 g soybean powder, 5 g peptone, 2 g $CaCO_3$, 8 g $MgSO_4{\cdot}7H_2O$, 6 g $FeSO_4{\cdot}7H_2O$, 2 g $ZnSO_4{\cdot}7H_2O$, 2 g $MnSO_4{\cdot}H_2O$, 0.5 g $CoCl_2{\cdot}6H_2O$, 2 g $Na_2MoO_4{\cdot}2H_2O$, pH 7.0–7.2. The fermentation was carried out at 28 °C for 6 days and stirred at 100 r.p.m. with an aeration rate of 900 l of air per hour.

The fermentation broth (30 l) was centrifuged to separate mycelial cake and supernatant. The mycelial cake was extracted with MeOH (5 l) and the supernatant was subjected to a Diaion HP-20 resin (Mitsubushi Chemical, Tokyo, Japan) column eluting with 95% EtOH (5 l). The MeOH extract and the EtOH eluents were evaporated under reduced pressure to yield a mixture (33 g) at 50 °C. The mixture was chromatographed on a silica gel column (Qingdao Haiyang Chemical Group, Qingdao, China; 100–200 mesh), and successively eluted with a stepwise gradient of $CHCl_3$/MeOH (100:0–50:50, v/v) to obtain three fractions (Fr.1–Fr.3) based on the TLC profiles. The Fr.2 was subjected to another silica gel column eluted with $CHCl_3$/MeOH (95:5–60:40, v/v) to give one fraction (Fr.2–1). Fr.2–1 was further isolated by preparative HPLC (Shimadzu LC-8 A, Shimadzu-C18, 5 μm, 250 × 20 mm inner diameter; 20 ml min^{-1}; 220 /254 nm; Shimadzu, Kyoto, Japan) eluting with a stepwise gradient MeOH/H_2O (10–70%, v/v 30 min) to obtain five subfractions (Fr.2–1–1 to Fr.2–1–5) based on the retention time. Then, Fr.2–1–2 (t_R 12.1 min) was purified by semi-preparative HPLC (Agilent 1100, Zorbax SB-C18, 5 μm, 250 × 9.4 mm inner diameter; 1.5 ml min^{-1}; 220 nm; Agilent, Palo Alto, CA, USA) eluting with CH_3CN/H_2O (12:88, v/v) to obtain maoxianamide A (**1**) (t_R 29.0 min, 31 mg). Fr.2–1–3 (t_R 10.3 min) was also isolated by semi-preparative HPLC eluting with CH_3CN/H_2O (17:83, v/v) to obtain maoxianamide B (**2**) (t_R 18.6 min, 6.3 mg). ^1H and ^{13}C NMR spectra were measured with a Bruker DRX-400 (400 MHz for ^1H and 100 MHz for ^{13}C) spectrometer (Rheinstetten, Germany). The ESIMS and HRESIMS spectra were taken on a Q-TOF Micro LC-MS-MS mass spectrometer (Milford, MA, USA).

Maoxianamide A (**1**) was obtained as colorless oil with UV (EtOH) λ_{max} nm (log ε): 202 nm (4.32) and $[\alpha]_D^{25}$+44 (c 0.25, EtOH).

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It exhibited a molecular formula of $C_{17}H_{31}NO_6$, as deduced from the HRESIMS at *m/z* 368.2039 $[M+Na]^+$ (calcd as 368.2044 for $C_{17}H_{31}NO_6Na$) in combination with ^{13}C NMR data (Table 1). The IR spectrum of **1** showed absorption bands for hydroxyl (at 3389 cm^{-1}) and carbonyl (at 1653 cm^{-1}) groups. Analysis of ^1H NMR spectrum of **1** revealed the presence of three olefinic protons at δ_H 5.68 (1H, m), 5.68 (1H, d, $J=9.2$ Hz), 5.25 (1H, br d, $J=8.8$ Hz), three aliphatic oxygenated methine protons at δ_H 4.75 (1H, d, $J=8.9$ Hz), 3.66 (1H, m), 3.22 (1H, dd, $J=10.4$, 1.9 Hz), six downfield methylene protons at δ_H 3.49 (2H, d, $J=5.2$ Hz), 3.39 (2H, m), 2.92 (1H, dd, $J=5.5$, 6.1 Hz), 2.95 (1H, dd, $J=6.0$, 5.4 Hz), an olefinic methyl at δ_H 1.76 (3H, d, $J=1.2$ Hz), one aliphatic singlet methyl at δ_H 1.25 (3H, s), one aliphatic triplet methyl at δ_H 1.00 (3H, t, $J=7.4$ Hz), in addition to proton signals at δ_H 1.76 (1H, m), 1.64 (1H, m), 1.56 (1H, m) and 1.25 (1H, m). The ^{13}C NMR and DEPT135 spectra (Table 1) of **1** showed 17 resonances attributable to an amide carbonyl carbon at δ_C 176.3, three sp^2 methines at δ_C 136.9, 127.6 and 125.3, one sp^2 quaternary carbon at δ_C 141.5, three oxygenated methines at δ_C 81.1, 71.3 and 70.1, one oxygenated quaternary carbon at δ_C 76.0, one oxygenated methylene at δ_C 67.2, four methylenes at δ_C 37.2, 36.5, 34.0, 25.2 and three methyl carbons at δ_C 23.7, 23.7, 11.7. The ^1H–^1H COSY correlations (Figure 1) of H_2-1′/H_2-2′/H-3′/H_2-4′ in **1** established connectivity from H-1′ atom along the chain through to C-4′ atom. The correlations between H_2/H_3, $H_5/H_6/H_7$, $H_9/H_{10}H_{11}$ protons in the ^1H–^1H COSY spectrum indicated the three structural units of C-2–C-3, C-5–C-7 and C-9–C-11. The observed HMBC correlations (Figure 1) from H_3-12 to C-3, C-4, C-5, from H_3-13 to C-7, C-8, C-9 established the linkage of C-2–C-11. The amide carbonyl group was connected with C-2 by the HMBC corrections from H-2 and H-3 to C-1 (δ_C 176.3). The connection of C-1 and C-1′ through a NH group was evident from the correlation of H_2-1′ to C-1 in the HMBC spectrum, and the ^1H and ^{13}C chemical shifts of C-1′. Taken the molecular formula of $C_{17}H_{31}NO_6$ into account, five hydroxyl groups were situated at C-2, C-8, C-9, C-3′ and C-4′, respectively. On the basis of the above spectroscopic data, a gross structure of **1** was established, and the ^1H and ^{13}C resonances in **1** were assigned (Table 1). The NOESY correlation between H_3-12 and H-2 demonstrated the geometry of the C-3–C-4 double bond was *E*. The geometry of $\Delta^{6,7}$ was also assigned as *E* by the large coupling constant ($J=15.8$ Hz) between H-6 and H-7 in the ^1H NMR spectrum obtained in pyridine-d_5. The other stereochemistry of **1** remained unassigned.

Maoxianamide B (**2**) was isolated as colorless oil with UV (EtOH) λ_{max} nm (log ε): 201 nm (4.20) and $[\alpha]_D^{25}$+57 (*c* 0.30, EtOH). Its molecular formula was determined to be $C_{17}H_{31}NO_6$ on the basis of the HRESIMS at *m/z* 346.2222 $[M+H]^+$ (calcd for $C_{17}H_{32}NO_6$ 346.2224). The IR spectrum of **2** showed absorption bands for hydroxyl (at 3364 cm^{-1}) and carbonyl (at 1650 cm^{-1}) groups. The ^1H NMR spectrum of **2** display two olefinic protons (δ_H 5.45 (1H, d, $J=8.4$ Hz), 5.27 (1H, d, $J=9.2$ Hz)), four aliphatic oxygenated methine protons (δ_H 4.79 (1H, d, $J=9.2$ Hz), 4.79 (1H, m), 4.59 (1H, m), 3.85 (1H, t, $J=6.8$ Hz)), six downfield methylene protons (δ_H 3.46 (2H, d, $J=5.9$ Hz), 3.34 (2H, m), 2.12 (1H, dd, $J=13.6$, 3.8 Hz), 2.59 (1H, dd, $J=13.6$, 9.1 Hz)), two olefinic methyls (δ_H 1.83 (3H, d, $J=0.9$ Hz), 1.68 (3H, d, $J=1.1$ Hz)), one aliphatic triplet methyl (δ_H 0.87 (3H, t, $J=7.6$ Hz)), as well as three proton signals (δ_H 1.76 (1H, m), 1.59 (1H, m), 1.52 (1H, m)). The ^{13}C NMR and DEPT135 spectra (Table 1) of **2** exhibited 17 carbon signals composing an amide carbonyl carbon (δ_C 176.1), two sp^2 methines (δ_C 130.3, 127.3), two sp^2 quaternary carbons (δ_C 139.9, 139.1), four oxygenated methines (δ_C 79.4, 71.2, 69.7, 67.1), one oxygenated methylene (δ_C 67.2), four methylenes (δ_C 41.6, 37.3, 33.9, 28.7) and three methyl carbons (δ_C 24.2, 12.0, 10.6). Comparison of the ^1H and ^{13}C NMR data of **2** with those of **1** suggested that **2** has the same skeleton as **1**. The differences between **2** and **1** were that the C-7

Table 1 ^1H and ^{13}C NMR data of maoxianamides A (1) and B (2)

Position	δ_H (*J* in Hz)			δ_C (p.p.m.)	
	1 (in CD_3OD)	**1** (in pyridine-d_5)	**2** (in CD_3OD)	**1** (in CD_3OD)	**2** (in CD_3OD)
1				176.3 (s)	176.1 (s)
2	4.75 d (8.9)	5.45 d (8.6)	4.79 d (9.2)	70.1 (d)	69.7 (d)
3	5.25 d (8.9)	5.80 d (8.6)	5.27 d (9.2)	125.3 (d)	127.3 (d)
4				141.5 (s)	139.1 (s)
5	2.92 dd (14.2, 6.1)	3.23 d (6.5)	2.12 dd (13.6, 3.8)	36.5 (t)	41.6 (t)
	3.01 dd (14.2, 6.0)		2.59 dd (13.6, 9.1)		
6	5.68 m	6.25 m	4.59 m	127.6 (d)	67.1 (d)
7	5.69 m	6.16 d (15.8)	5.45 d (8.4)	136.9 (d)	130.3 (d)
8				76.0 (s)	139.9 (s)
9	3.22 dd (10.4, 1.9)	3.77 d (10.1)	3.85 t (6.8)	81.1 (d)	79.4 (d)
10	1.25 m	1.68 m	1.55 m	25.2 (t)	28.7 (t)
	1.64 m	2.05 m			
11	1.00 t (7.4)	1.26 t (7.3)	0.88 t (7.4)	11.7 (q)	10.6 (q)
12	1.76 d (1.2)	1.76 br s	1.83 d (0.9)	23.7 (q)	24.2 (q)
13	1.25 s	1.65 s	1.68 d (1.1)	23.7 (q)	12.0 (q)
1′	3.39 m	3.87 m	3.34 m	37.2 (t)	37.3 (t)
		4.03 m			
2′	1.56 m	2.01 m	1.52 m	34.0 (t)	33.9 (t)
	1.76 m	2.19 m	1.76 m		
3′	3.66 m	4.30 m	4.79 m	71.3 (d)	71.2 (d)
4′	3.49 d (5.2)	4.03 d (5.9)	3.46 d (5.9)	67.2 (t)	67.2 (t)

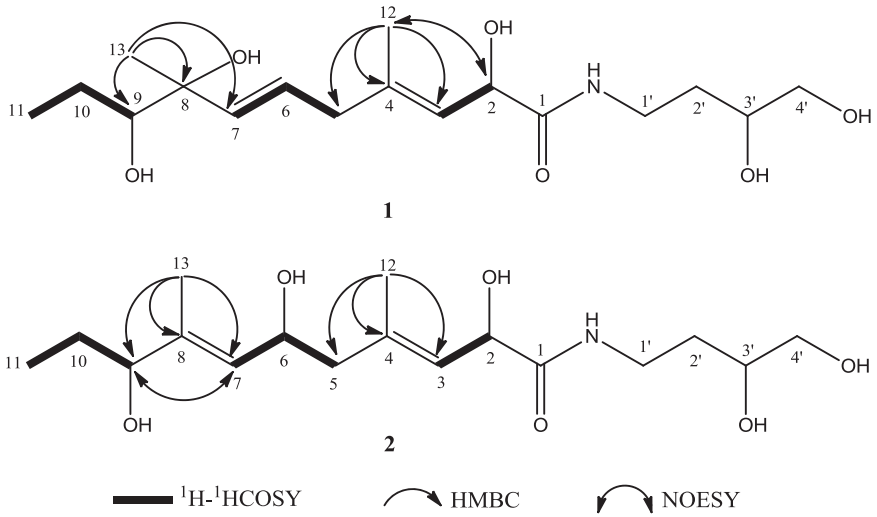

Figure 1 Structures and key ^{1}H–^{1}H COSY, HMBC and NOESY correlations of maoxianamides A (**1**) and B (**2**).

hydroxyl group and $\Delta^{6,7}$ olefin in **1** were replaced by the C-6 hydroxyl group and $\Delta^{7,8}$ olefin in **2**. The correlation of H-6/H-7 in the ^{1}H–^{1}H COSY spectrum (Figure 1) and the observed HMBC correlated signals from H_3-13 to C-7, C-8, C-9 further confirmed the assignment. The geometry of $\Delta^{7,8}$ was assigned as *E* by the NOESY correlations between H-9 and H-7. The other relative stereochemistry of **2** was assigned by analogy with **1**.

The antimicrobial activities of maoxianamides A (**1**) and B (**2**) using a disk diffusion assay was carried out against five microorganisms.[10] Neither maoxianamides A (**1**) nor B (**2**) were found to be active against *Micrococcus luteus*, *Bacillus subtilis*, *Candida albicans* and *Rhizoctonia solani*, even at 100 μg per 7 mm paper disks. Maoxianamides A (**1**) and B (**2**) exhibited weakly inhibitory activity against *Sclerotinia sclerotiorum* with inhibition zones of 7 mm and 9 mm at 100 μg per 7 mm paper disks.

The cytotoxicity of maoxianamides A (**1**) and B (**2**) were assayed *in vitro* against the human lung carcinoma A549 cell line, human hepatoma carcinoma HepG2 cell line and human leukemia K562 cell line by the CCK8 method as described in our previous papers.[11,12] Maoxianamide A (**1**) exhibited cytotoxic activity with IC_{50} (half maximal inhibitory concentration) values of 55.3, 32.4 and 33.2 μg ml^{-1}, respectively. The values of maoxianamide B (**2**) were 60.4, 37.7 and 40.6 μg ml^{-1}.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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