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Drimane sesquiterpenoids from a wetland soil-derived fungus *Aspergillus calidoustus* TJ403-EL05

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Abstract

Soil-derived fungi represent an insufficiently tapped reservoir for discovering new and bioactive natural products (NPs), and despite an ever-increasing number of unknown NPs have been discovered over the past few decades, much of the hidden biosynthetic potential is still in an urgent need to be disclosed. In this research, a chemical investigation was performed on a wetland soil-derived fungus *Aspergillus calidoustus* TJ403-EL05, leading to the isolation of a total of fourteen drimane sesquiterpenoids (**1**–**14**), incorporating three new ones, namely ustusols F–H (**1**–**3**). Their structures, comprising absolute configurations, were completely authenticated by widespread spectroscopic data, quantum chemical ^{13}C NMR and ECD calculations, and X-ray crystallography experiments. Compound **14** exhibited moderate anti-inflammatory activity by inhibiting the LPS-induced NO release ($IC_{50} = 25.6$ μM).

Keywords: *Aspergillus calidoustus*, Drimane sesquiterpenoids, Structure elucidation, Anti-inflammatory activity

[†]Sitian Zhang and Shuyuan Mo have equally contributed to this work.

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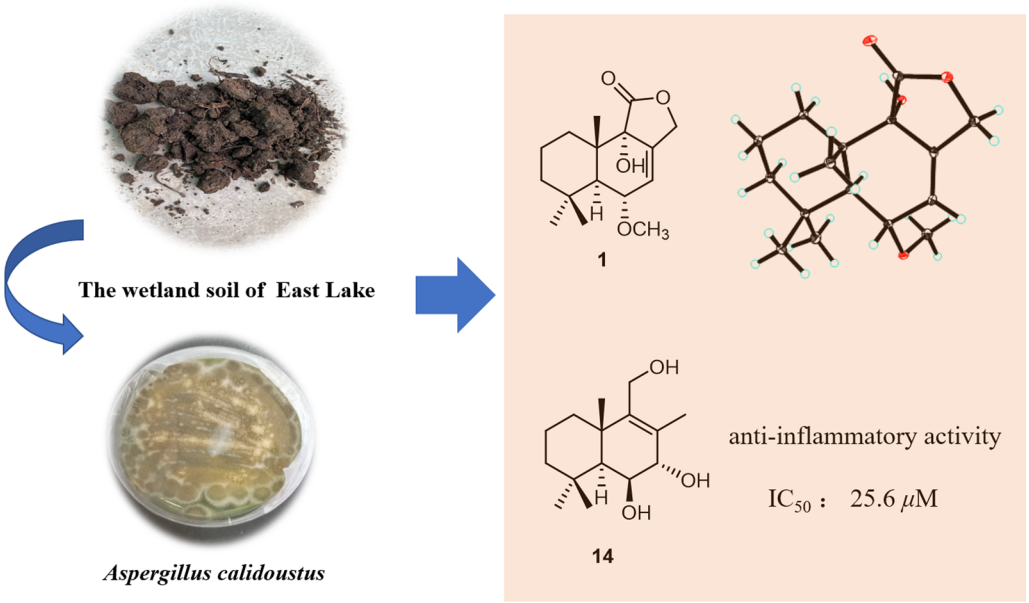
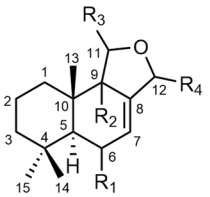
Graphical Abstract

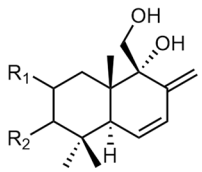


1 Introduction

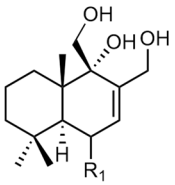
Over the past few decades, a large proportion of medicines originate from various natural resources, especially from the field of microbiology [1]. Terrestrial microorganisms have a huge biosynthetic capacity to produce structurally diverse and pharmacologically active NPs, which have become important chemical entities in drug discovery [2]. For example, cyclosporine, isolated from the soil-derived *Tolypocladium inflatum*, was the first immunosuppressive agent to enable selective immune



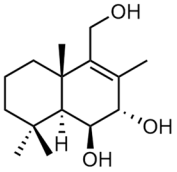
	R_1	R_2	R_3	R_4
1	α–OCH_3	α–OH	=O	H
2	α–OH	α–OH	H	H
4	α–OH	α–OH	α–OH	H
5	α–OCH_3	α–OH	α–OH	H
6	=O	α–OH	=O	H
7	=O	α–OH	α–OH	H
8	β–OH	α–OH	=O	H
9	β–OH	α–OH	H	=O
10	α–OH	α–H	α–OH	H



	R_1	R_2
3	H	β–OH
11	α–OH	H



	R_1
12	=O
13	α–OH



14

Fig. 1 Chemical structures of compounds **1–14**

regulation of T cells, without excessive toxicity [3]. The discovery of cyclosporine in 1971 initiated a new era in the immunopharmacology field, and is still widely used in clinical practice. Therefore, soil-derived fungi have attracted, and will attract increasing attention in the NPs-related research fields.

Aimed at searching for structurally unique and pharmacologically attractive NPs from the soil-derived fungi [4–6], strain *A. calidoustus* TJ403-EL05 that was separated from a wetland soil collected from East Lake in Wuhan City, caught our attention and was thus chemically investigated, which afforded three new drimane sesquiterpenoids, namely ustusols F–H (**1**–**3**), and eleven known congeners (**4**–**14**). In this paper, the isolation, structural characterization, and anti-inflammatory activity of these drimane sesquiterpenoids (Fig. 1) were elaborated.

2 Results and discussion

Compound **1** was purified as a colorless crystal. According to the HRESIMS analysis showing a sodium adduct ion at *m*/*z* 303.1567 (calcd for 303.1567), its molecular formula was determined as $C_{16}H_{24}O_4$, implying 5 degrees of unsaturation. The ^{1}H NMR data (Table 1) of **1** showed obvious signals as three methyl protons (δ_H 1.08, 1.04, and 0.90), four methylene protons (δ_H 4.97/4.70, 1.62/2.08, 1.42/1.33, and 1.58), three methine protons (δ_H 6.05, 3.97, and 1.98) and one methoxy proton (δ_H 3.31). With the help of DEPT and HSQC spectroscopic analyses, the ^{13}C NMR data (Table 1) of **1** demonstrated the existence of 16 carbon signals that were attributable to three methyls (δ_C 35.0, 23.1 and 18.5), one methoxy (δ_C 54.4), four methylenes (δ_C 69.0, 43.1, 30.5, and 18.4), three methines (δ_C 125.2, 76.3, and 46.1), four quaternary carbons (δ_C 135.6, 74.6, 41.7 and 33.8) and one ester carboxyl carbon (δ_C 175.2). The 1D and 2D NMR data of **1** were highly similar to those of the known 9*α*-hydroxy-5*α*-drim-7-ene-6-one-11,12-olide (**6**) [7], uncovering **1** and **6** to possess the same drimane sesquiterpenoid core skeleton. The significant difference of **1** and **6** was the existence of one

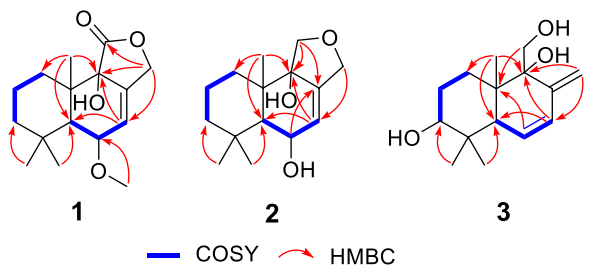


Fig. 2 Key ^{1}H–^{1}H COSY and HMBC correlations of compounds **1**–**3**

Table 1 NMR data of **1**–**3** (*δ* in ppm, *J* in Hz)

No.	1 (In $CDCl_3$)		2 (In DMSO-d_6)		3 (In $CDCl_3$)	
	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[b]
1	1.62 m; 2.08 m	30.5 CH_2	1.39 m; 1.61 m	32.3 CH_2	1.43 m; 1.74 m	29.5 CH_2
2	1.58 m	18.4 CH_2	1.39 m; 1.45 m	18.3 CH_2	1.62 m; 1.74 m	27.6 CH_2
3	1.33 m; 1.42 m	43.1 CH_2	1.14 m; 1.26 m	43.3 CH_2	3.33 m	78.2 CH
4		33.8 C		32.9 C		38.6 C
5	1.98 d (9.0)	46.1 CH	1.71 d (10.2)	48.3 CH	2.44 t (3.0, 1.7)	46.8 CH
6	3.97 m	76.3 CH	4.02 m	67.1 CH	5.79 dd (10.2, 1.7)	129.1 CH
7	6.05 m	125.2 CH	5.65 d (1.7)	131.9 CH	6.11 dd (10.2, 3.0)	129.5 CH
8		135.6 C		138.2 C		145.6 C
9		74.6 C		74.6 C		76.1 C
10		41.7 C		42.0 C		41.3 C
11		175.2 C	3.45 d (14.5)	62.0 CH_2	3.82 d (11.0); 3.93 dd (11.0, 4.7)	62.1 CH_2
12	4.70 dt (12.4, 2.0); 4.97 dt (12.4, 2.0)	69.0 CH_2	4.04 d (11.0)	61.5 CH_2	5.03 d (1.4); 5.48 d (1.4)	114.8 CH_2
13	0.90 s	18.5 CH_3	0.90 s	17.5 CH_3	0.75 s	16.4 CH_3
14	1.04 s	23.1 CH_3	1.00 s	22.9 CH_3	0.84 s	16.7 CH_3
15	1.08 s	35.0 CH_3	1.10 s	36.6 CH_3	1.10 s	28.2 CH_3
OCH_3	3.31 s	54.4 CH_3				

[a] Recorded at 400 MHz

[b] Recorded at 100 MHz

"m" means overlapped or multiplet with other signals

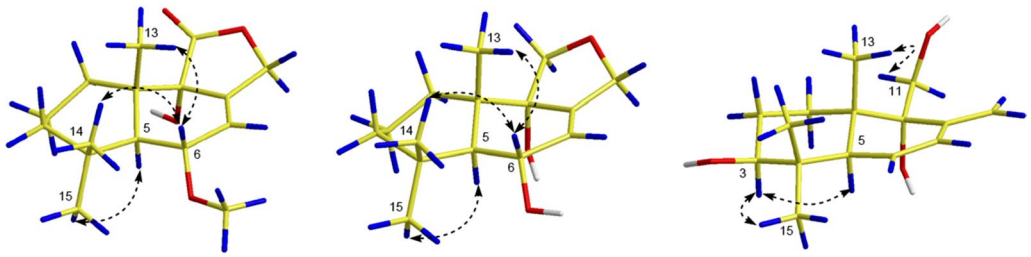


Fig. 3 Key NOESY correlations (dashed black arrows) of compounds **1**–**3**

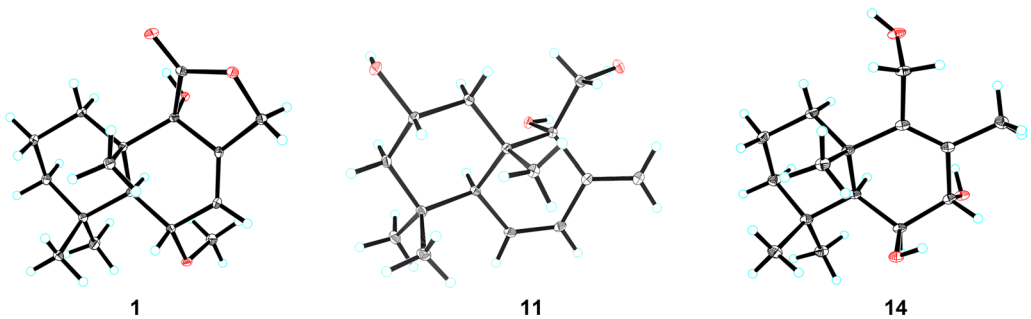


Fig. 4 X-ray crystallographic structures of **1**, **11**, and **14**

methoxy group linked at C-6 in **1** instead of a conjugated ketone carbonyl (C-6) in **6**, as further supported based on the key HMBC correlations (Fig. 2) of 6-OMe (δ_H 3.31) with C-6 (δ_C 76.3) and of H-6 (δ_H 3.97) with C-5 (δ_C 46.1), C-7 (δ_C 125.2), and C-8 (δ_C 135.6). In the NOESY experiment (Fig. 3), the key NOE correlations of H-6 with Me-14 (δ_H 1.04)/Me-13 (δ_H 0.90) and of H-5 (δ_H 1.98) with Me-15 (δ_H 1.08) suggested that H-6, Me-13 and Me-14 should all be β-oriented, while H-5 and Me-15 were all α-oriented. However, no useful NOE signals could be applied to verify the configuration of C-9. Fortunately, a suitable crystal of **1** was acquired by recrystallization and then furnished for X-ray crystallographic experiment (Fig. 4). According to a Flack parameter of 0.01(3), the absolute configuration of **1** was unequivocally confirmed as 5*S*, 6*S*, 9*S*, and 10*S*. Accordingly, the absolute structure of **1**, named as ustusol F, was defined.

Compound **2**, obtained as a white powder, was determined to possess a molecular formula of $C_{15}H_{24}O_3$, as evidenced by its positive HRESIMS data at *m/z* 275.1618 (calcd for $C_{15}H_{24}O_3Na^+$, 275.1618). By comparing its ^1H, ^{13}C, and DEPT NMR data (Table 1) with those of the known 6-*epi*-pereniporin A (**4**) [8], we could speculate that both compounds were structural analogues, with the only distinction being that one hydroxy group linked at C-11 was absent in **2**, as fully supported by the HMBC

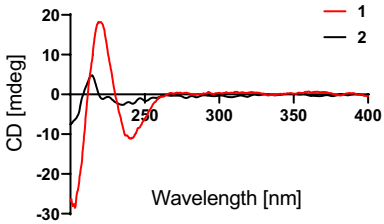


Fig. 5 Experimental ECD curves of compounds **1** and **2** in MeOH

correlations (Fig. 2) of H_2-11 (δ_H 3.45) with C-8 (δ_C 138.2), C-9 (δ_C 74.6), and C-12 (δ_C 61.5). Similar NOESY data (Fig. 3) and ECD curves (Fig. 5) between **1** and **2** proved that these two compounds possessed the identical absolute configuration. Accordingly, the absolute structure of **2**, named as ustusol G, was defined.

Compound **3** was deduced to have a molecular formula of $C_{15}H_{24}O_3$, as evidenced via its HRESIMS data. By comparing the 1D NMR data (Table 1) of **3** to those of the known ustusol D (**11**) [9] (Fig. 1) whose absolute structure was verified via crystallography experiment (Fig. 4), it revealed that both compounds possessed the identical drimane sesquiterpenoid core skeleton, with the only exception that a hydroxy group was linked at C-2 in **11** by C-3 in **3**. This conclusion was further corroborated via the key ^1H–^1H COSY cross-peaks of

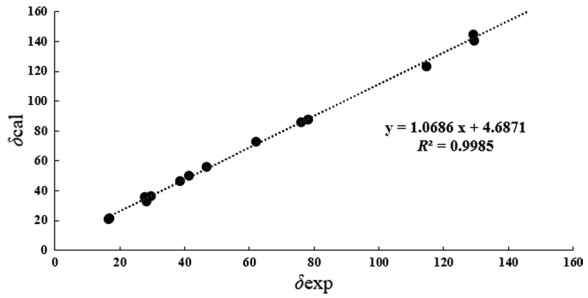


Fig. 6 Linear correlation between the experimental and calculated ^{13}C NMR chemical shifts for **3**

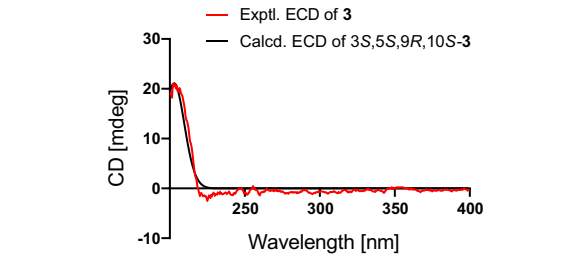


Fig. 7 Experimental and calculated ECD spectra of compound **3**

H_2-1/H_2-2/H-3, as well as the HMBC correlations of both Me-14 and Me-15 with C-3, C-4, and C-5 (Fig. 2). The NOE cross-peaks (Fig. 3) of Me-15α/H-3/H-5 and H_2-11/Me-13β demonstrated that OH-3 was β-oriented in **3**. To validate this speculation, the ^{13}C NMR chemical shifts of **3** were predicted at the B972/pcSseg-2 level showing the correlation coefficient (R^2) value of 0.9985 (Fig. 6), which completely supported our proposed relative structure. Lastly, the quantum chemical electronic circular dichroism (ECD) calculation was employed for **3**. To our expectation, the calculated ECD plot was closely similar to the experimental one (Fig. 7), proclaiming its absolute configuration as 3*S*, 5*S*, 9*R*, and 10*S*, and this compound was named as ustusol H.

Apart from new compounds **1**–**3**, eleven known congeners were also isolated from *A. calidoustus* TJ403-EL05 and identified as 6-*epi*-pereniporin A (**4**) [8], 6-*epi*-*O*-methyl-pereniporin A (**5**) [8], 9*a*-hydroxy-5*a*-drim-7-ene-6-one-11,12-olide (**6**) [7], 6-dehydroxy-6-oxopereniporin A (**7**) [8], strobilactone A (**8**) [10], pereniporin B (**9**) [11], dendocarbin C (**10**) [12], ustusol D (**11**) [9], 9*a*,11,12-trihydroxydrim-7-en-6-one (**12**) [13], 12-hydroxyalbrassitriol (**13**) [14] and drim-8-en-6*β*,7*a*,11-triol (**14**) [15], by comparison of their HRESIMS and NMR data with those reported in the literature.

In the bioactivity assay, due to the limited amounts of **3**, other compounds (**1**–**2** and **4**–**14**) were tested for anti-inflammatory activity by using LPS-induced murine macrophages RAW264.7 cells. As a result, only compound **14** was found to show an inhibitory effect against the NO release (IC_{50} = 25.6 μM), and the remaining compounds did not exhibit significant activity with IC_{50} values of > 40 μM (positive control MG132: IC_{50} = 0.32 μM).

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s13659-022-00349-w.

Additional file 1. Supplementary figures and tables.

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Author contributions

All authors read and approved the final manuscript.

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Declarations

Competing interests

The authors declare no conflict of interest.

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