The Journal of Antibiotics (2010) 63, 713–715

www.nature.com/ja

NOTE

Cytotoxic aromatic compounds from *Hericium erinaceum*

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The Journal of Antibiotics (2010) **63,** 713–715; doi:10.1038/ja.2010.112; published online 6 October 2010

Keywords: hericenone I; hericene D; *Hericium erinaceum*; mushroom

Hericium erinaceum is a mushroom belonging to the family Hericiaceae and has been used as medicine or food in China and Japan without harmful effects. *H. erinaceum* grows on old or dead broadleaf trees and has been used as medicine for treatment of gastricism in traditional Chinese medicine for more than 1000 years. Recently, the chemical constituents of *H. erinaceum* have been investigated for its interesting and significant bioactivities.[1,2] Hericenones A and B were isolated from the fruiting body of *H. erinaceum* as cytotoxic principles against Hela cells.[3] Hericenones C, D and E exhibited stimulating activity to the synthesis of nerve growth factor *in vitro*.[4–7] In the presence of hericenones C, D, E and H at 33 μg ml^{-1}, mouse astroglial cells secreted 23.5 ± 1.0, 10.8 ± 0.8, 13.9 ± 2.1 and 45.1 ± 1.1 pg per ml nerve growth factor into the culture medium, respectively. The degree of activity for hericenone D was almost at the same level as that of a potent stimulator, epinephrine. It is of interest that the difference in activity among those compounds was dependent on the nature of the fatty acid. These results suggest the usefulness of *H. erinaceum* for the treatment and prevention of dementia.

In the course of our continuing search for new active compounds from mushroom, we found two new aromatic compounds, namely hericenone I and hericene D. In this report, we described the isolation, structural elucidation and cytotoxic activity of hericenone I and hericene D (Figure 1).

The $CHCl_3$-soluble fraction of MeOH extract from the fruiting bodies of *H. erinaceum* was subjected to repeated chromatography to yield hericenone I and hericene D. Hericenone I was obtained as a white oil. The HRESI-MS of hericenone I gave an ion $[M+Na]^+$ peak at *m/z* 619.6686 (calc. for $C_{37}H_{56}O_6Na$ 619.6702) and corresponded to a molecular formula of $C_{37}H_{56}O_6$. The IR spectrum indicated the presence of a hydroxyl group at 3463 cm^{-1} and an α, β-unsaturated ketone (1669 cm^{-1}). The ^{13}C and DEPT-NMR spectrum of hericenone I was quite similar to that of hericenone D, except for the presence of two more olefinic CH groups (129.7, CH; 130.0, CH).[4] The ^1H NMR spectrum of hericenone I showed the signals of three methines at δ 5.37 (1H, t, *J*=7.2 Hz), 6.15 (1H, s) and δ 6.54 (1H, s); four methyls at δ 0.86 (3H, t, *J*=7.0 Hz), δ 1.78 (3H, s), δ 1.83 (3H, s) and δ 2.16 (3H, s); and one methoxyl at δ 3.91 (3H, s), together with one CHO group at δ 10.20 (1H, s) (Table 1). Methanolysis of hericenone I gave the methyl ester of oleic acid, which was identified by GC-MS. Considering the molecular formula of hericenone I, it was supposed to be an oleic ester of hericenone. The construction of molecular framework was deduced from the ^1H–^1H COSY, heteronuclear multiple quantum coherence and heteronuclear multiple bond coherence spectra (Figure 2).

Hericene D was also obtained as a white oil. The HRESI-MS of hericene D gave an ion $[M+Na]^+$ peak at *m/z* 603.4026 (calc. for $C_{37}H_{56}O_5Na$ 603.4031) and corresponded to a molecular formula of $C_{37}H_{56}O_5$, requiring 10 degrees of unsaturation. The IR spectrum indicated the presence of a hydroxyl group at 3446 cm^{-1}. The ^{13}C and DEPT-NMR spectrum of hericene D was similar to that of hericene A, except for the presence of four more olefinic CH groups (δ 127.9, CH; 128.1, CH; 130.0, CH; 130.2, CH).[6] The ^1H NMR spectrum of hericene D showed the signals of three methines at δ 5.05 (1H, t, *J*=7.0 Hz), 5.17 (1H, t, *J*=8.0) and δ 6.53 (1H, s); four methyls at δ 0.87 (3H, t, *J*=7.0 Hz), δ 1.60 (3H, s), δ 1.63 (3H, s) and δ 1.77 (3H, s); and one methoxyl at δ 3.91 (3H, s), together with one CHO group at δ 10.10 (1H, s) (Table 2). Methanolysis of hericene D yielded the methyl ester of linoleic acid, which was also identified by GC-MS. Considering the molecular formula, hericene D was deduced to be a linoleic ester of hericene.

Compounds **1–5** were tested *in vitro* for cytotoxic activity against tumor cells by MTT assay. All the compounds showed cytotoxic activity against EC109 cell line, and the cell growth inhibitor percentages of compounds **1–5** on EC109 cell line are shown in Table 3.

It may be pointed out that hericenone I and hericene D have a structure related to mycophenolic acid, an antibiotic produced by *Penicillium* spp.[6] Hericenone and hericene D were both tested against the bacteria, *Staphylococcus aureus*, *Bacillus thuringiensis*, *Escherichia coli*, *Bacillus megaterium* and *Bacillus subtilis* at 1 mg ml^{-1}, respectively. However, hericenone I and hericene D showed only weak antibacterial

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Received 6 June 2010; revised 16 August 2010; accepted 18 August 2010; published online 6 October 2010

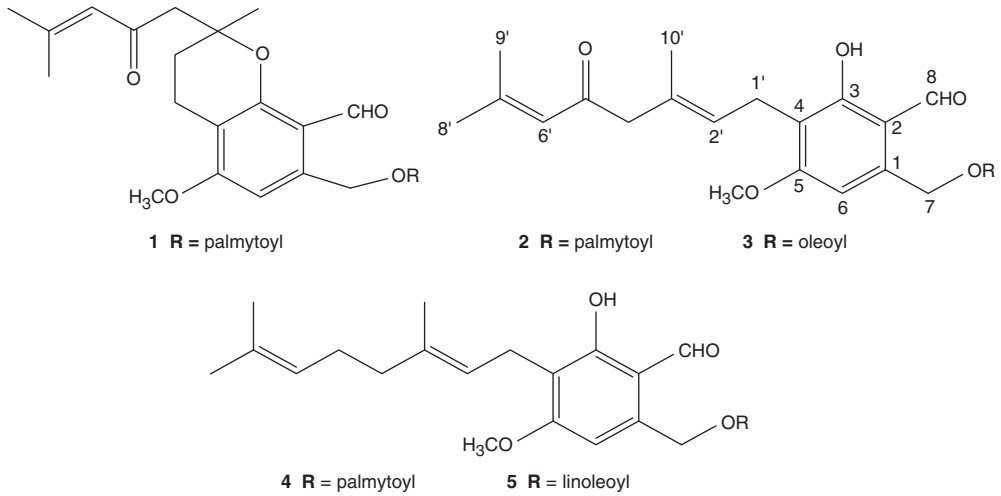

Figure 1 Structures of compounds **1–5**.

Table 1 ^{1}H and ^{13}C NMR data of herienone I in $CDCl_3$

Position	*δ C*	*δ H*
1	138.7	
2	112.9	
3	162.9	
4	117.3	
5	163.5	
6	105.6	6.54 (1H, s)
7	62.9	5.18 (2H, s)
8	193.1	10.20 (1H, s)
1′	21.6	3.90 (2H, d, 7.2)
2′	126.3	5.37 (1H, t, 7.2)
3′	130.2	
4′	55.6	3.02 (2H, s)
5′	199.6	
6′	122.3	6.15 (1H, s)
7′	155.4	
8′	27.5	1.83 (3H, s)
9′	20.6	2.16 (3H, s)
10′	16.4	1.78 (3H, s)
1″	173.1	
2″	34.2	2.35 (2H, t, 7.6)
9″, 10″	129.7, 130.0	5.34 (2H, m)
18″	14.1	0.86 (3H, t, 6.8)
5-OCH_3	55.9	3.91 (3H, s)

Table 2 ^{1}H and ^{13}C NMR data of hericene D in $CDCl_3$

Position	*δ C*	*δ H*
1	138.4	
2	112.9	
3	162.9	
4	118.1	
5	163.6	
6	105.6	6.53 (1H, s)
7	62.9	5.35 (2H, s)
8	193.1	10.10 (1H, s)
3-OH		12.37 (1H, s)
1′	21.4	3.34 (2H, d, 8.0)
2′	121.2	5.17 (1H, t, 8.0)
3′	131.2	
4′	39.8	1.97 (2H, m)
5′	26.7	2.04 (2H, m)
6′	124.4	5.05 (1H, t, 7.0)
7′	135.7	
8′	25.6	1.63 (3H, s)
9′	17.6	1.60 (3H, s)
10′	16.1	1.77 (3H, s)
1″	173.1	
2″	34.2	2.33 (2H, t, 8.0)
9″, 10″, 12″, 13″	127.9, 128.1, 130.0, 130.2	5.37 (4H, m)
18″	14.1	0.87 (3H, t, 7.0)
5-OCH_3	55.9	3.91 (3H, s)

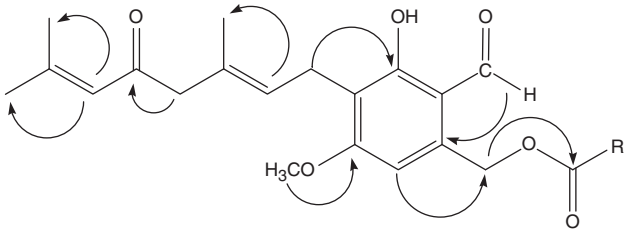

Figure 2 Key HMBC correlations of hericenone I.

activity *in vitro* against the five bacteria above. The damage of the γ-lactone ring in the structures of hericenone I and hericene D may be the main reason for the deficiency of antibacterial activity.

EXPERIMENTAL PROCEDURE

General

IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets (Bio-Rad, Philadelphia, PA, USA). NMR spectra were recorded on a Brucker AV-400 spectrometer (Bruker Biospin, Fällanden, Switzerland) in $CDCl_3$ solvent, with tetramethylsilane as the internal standard. MS were recorded on

Table 3 Cell growth inhibitor percentages of compounds 1–5 on EC109 cell line

Compounds	*Concentration (mol l^{-1})*	*Cell growth inhibitor (%)*
1	1×10^{-3}	65.34
	3×10^{-4}	36.68
	1×10^{-4}	33.75
2	1×10^{-3}	57.75
	3×10^{-4}	34.97
	1×10^{-4}	28.58
3	1×10^{-3}	59.23
	3×10^{-4}	48.06
	1×10^{-4}	41.33
4	1×10^{-3}	58.22
	3×10^{-4}	42.18
	1×10^{-4}	38.09
5	1×10^{-3}	50.12
	3×10^{-4}	45.66
	1×10^{-4}	32.27

VG Auto-spec-3000 (VG, Manchester, UK) and API QSTAR Plusar spectrometer (Applid Biosystems, Foster City, CA, USA).

Silica gel (200–300 mesh, Qingdao Marine Chemical, Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used for column chromatography. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol.

Mushroom material

The fresh fruiting bodies of *H. erinaceum* were collected on Funiu Mountain of He'nan Province, People's Republic of China, in July 2008. The fungal identification was made by Professor Jin-Wen Shen, Henan Agricultural University. A dried specimen was deposited in the Herbarium of Henan Agricultural University.

Extraction and isolation

The air-dried and powdered fruiting bodies of *H. erinaceum* (dry weight 2.0 kg) were extracted with MeOH at room temperature to afford a deep brown gum (225 g). Then the MeOH extract was diluted with H_2O and partitioned with $CHCl_3$. The $CHCl_3$ extract (62 g) was subjected to column chromatography eluting with petroleum ether/EtOAc. The fraction (0.9 g) from petroleum ether/EtOAc (20 : 1, v/v) was submitted for further purification by repeated Sephadex LH-20 ($CHCl_3$/MeOH=1 : 1, v/v) to give hericenone F (85 mg). The fraction (1.2 g) from petroleum ether/EtOAc (19 : 1, v/v) was submitted for further purification by repeated Sephadex LH-20 ($CHCl_3$/MeOH=1 : 1, v/v) to give a mixture of hericenones C and I. Preparative HPLC with ODS column (250×30 mm, 5 ml min^{-1}, MeOH/H_2O=96 : 4, detection at 298 nm) to give hericenones C (56 mg) and I (18 mg).

The fraction (1.6 g) from petroleum ether/EtOAc (10 : 1, v/v) was submitted for further purification by repeated Sephadex LH-20 ($CHCl_3$/MeOH=1 : 1, v/v) to give a mixture of hericenes A and D. Preparative HPLC with ODS column (250×30 mm, 5 ml min^{-1}, MeOH/H_2O=95 : 5, detection at 295 nm) to give hericenes A (150 mg) and D (35 mg).

Hericenone I. White oil: UV λ_{max} ($CHCl_3$) 301 nm. IR (KBr) 3463, 2918, 1742, 1669 and 1567 cm^{-1}. *^{1}H and ^{13}C NMR spectra*: see Table 1. HRESI-MS *m/z* 619.6686 (M+Na, calc. for $C_{37}H_{56}O_6Na$ 619.6702).

Hericene D. White oil: UV λ_{max} ($CHCl_3$) 296 nm. IR(KBr) 3446, 2923, 1754 and 1571 cm^{-1}. *^{1}H and ^{13}C NMR spectra*: see Table 2. HRESI-MS *m/z* 603.4026 (M+Na, calc. for $C_{37}H_{56}O_5Na$ 603.4031).

Methanolysis of Hericene D and Hericenone I

Hericenone I (5 mg) and Hericene D (5 mg) were dissolved with 0.1 M methanolic KOH (5 ml) and stirred overnight at 4 °C. Water was added to the reaction mixture, and the solution was neutralized with 0.1 M HCl and then extracted with hexane. The hexane layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give the methyl ester of oleic acid and the methyl ester of linoleic acid, which were identified by GC-MS.

Cell growth inhibition assay

The growth inhibitor concentrations of compounds **1–5** on esophageal cancer EC109 cell line were determined by MTT assay.[8] The EC109 cell was exposed to compounds at 1×10^{-3}, 3×10^{-4} and 1×10^{-4} mol l^{-1} and each concentration was tested in triplicate. The OD was measured with a microplate reader at 570 nm.

ACKNOWLEDGEMENTS

This project was supported by the National Natural Science Foundation of China (30901957) and Program for Excellent Young Teachers of He'nan Province.

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