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BRIEF COMMUNICATION

Three new cyathane diterpenes with neurotrophic activity from the liquid cultures of *Hericium erinaceus*

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Abstract

Three new cyathane diterpenes erinacines T–V (**1**–**3**), and two known cyathane diterpenes erinacine A (**4**) and erinacine P (**5**) were isolated from the liquid cultures of *Hericium erinaceus*. The structures of **1**–**3** were determined by extensive spectroscopic analysis. All isolated compounds were evaluated for the cytotoxicity, and neurite-promoting activities using PC12 cell line. Compounds **1**–**3**, and **5** exhibited pronounced neurite outgrowth-promoting effects on PC12 cells in the range of 2.5−10 μM. Compound **4** showed weak cytotoxicity against PC12 cells with IC_{50} of 73.7 μM.

Hericium erinaceus, a well-known edible and medicinal mushroom, has a long history of being used as healthy food in China and Japan [1]. The fruiting bodies or mycelia of *H. erinaceus* have been demonstrated with a wide range of beneficial activities, such as hypolipidemic, anticancer, antihypertensive, and neuronal disease-protecting activities [2, 3]. Previous chemical researches on *H. erinaceus* have revealed a number of bioactive secondary metabolites with diverse chemical skeletons, such as pyrones (erinapyrones A and B) [4], sesterterpene (erinacine S) [5], meroterpenoid (herienones C–E) [6], and cyathae-type diterpenes (erinacines A–K and P–R) [7–11].

Cyathane diterpenes represent a group of secondary metabolites with an unusual 5/6/7 tricyclic skeleton from basidiomycota. A number of cyathane diterpenes have been reported from mushrooms in the genera of *Cyathus*, *Hericium*, and *Sarcodon*, and proven to exhibit a wide range of interesting and significant biological activities [12, 13]. Cyathins Q and R from *C. africanus* exhibited potent apoptosis-inducing effects on HCT116 cell line and further in vivo anticancer activities in rats [14, 15]. Erinacines from *H. erinaceus* and scabronines from *S. scabrosus* displayed stimulating effect on the synthesis of nerve growth factor (NGF) in human nerve cells [7–11, 16]. Striatoids A–F from *C. striatus* dose-dependently enhanced NGF-mediated neurite outgrowth in rat pheochromocytoma (PC12) cells [17]. Recently, erinacine A, a cyathane-type diterpene isolated from the mycelia of *H. erinaceus*, was confirmed to possess a beneficial effect on increasing the content of catecholamine and NGF in the central nervous system of rats, which implicates its potential as therapeutic agents to treat neurodegenerative ailments such as Alzheimer's or Parkinson's disease [18].

In our searching for neurotrophic natural products from the medicinal mushrooms, chemical investigations on the liquid cultures extract from *H. erinaceus* led to the isolation of three new cyathane diterpenes (**1**–**3**) and two known compounds (**4** and **5**). In this paper, we report the isolation, structure determination, and neurotrophic effects on PC12 cells of **1**–**5** (Fig. 1).

The ethyl acetate extract of *H. erinaceus* fermented on HY culture medium was isolated to afford five cyathane diterpenes by comprehensive chromatography methods. Two known compounds were identified as erinacine A (**4**) [9] and erinacine P (**5**) [11] by comparing their nuclear magnetic resonance (NMR) and mass spectrometry (MS) data with previously reported data. The structures of new

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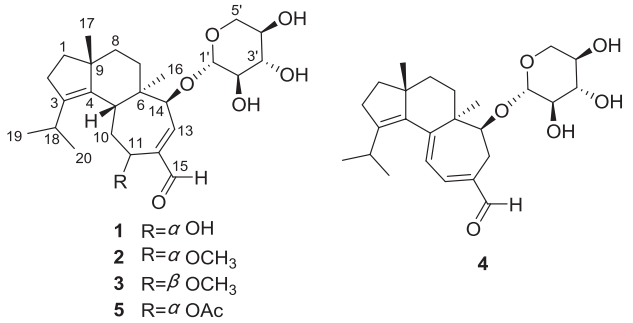

Fig. 1 Structures of compounds **1**–**5**

compounds erinacines T–V (**1**–**3**) were assigned by extensive spectroscopic analysis.

Erinacine T (**1**) was obtained as white powder. Its molecular formula of $C_{25}H_{38}O_7$ was established on the basis of the electron spray ionization (ESI) high-resolution MS (HRMS) ion at *m/z* 473.2511 $[M + Na]^+$ (calcd for $C_{25}H_{38}O_7Na$, 473.2510). The ^1H, ^{13}C NMR (Table 1), and heteronuclear single quantum coherence spectroscopy data displayed four methyls, six methylenes containing one oxygenated methylene, eight methines including six oxygenated methines, two pairs of olefinic carbons, one aldehyde group, two sp^3 quaternary carbons, and four hydroxyl protons. These data suggested that **1** has a similar structure as that of erinacine P (**5**). The key HMBC correlations from H_2-1 to C-3, C-4, C-8, C-9, and C-17; from H_2-2 to C-3, C-4, and C-9; from H-5 to C-3, C-4, C-6, C-7, and C-11; from H_2-7 to C-6, C-9, and C-16; from H_2-8 to C-4, C-6, C-9, and C-17; from H_2-10 to C-6 and C-12; from H-11 to C-12, C-13, and C-15; from H-13 to C-11, C-12, C-6, and C-15; from H-14 to C-6 and C-12; from H-15 to C-11, C-12, and C-13; from H_3-16 to C-6, C-7, and C-14; from H_3-17 to C-1, C-4, C-8, and C-9; from H-18 to C-2, C-3, and C-4; from H_3-19(20) to C-3 and C-18, along with the ^1H-^1H COSY correlations of H_2-1/H_2-2, H_2-7/H_2-8, H_2-10/H-11, H-11/OH-11, H-13/H-14, and H-18/H_3-19(20) indicated the cyathane diterpene unit in **1** (Figure S1). The ^1H-^1H COSY correlations of H-1′/H-2′/H-3′/H-4′/H_2-5′, H-2′/OH-2′, H-3′/OH-3′, and H-4′/OH-4′, and key HMBC correlation from H-1′ to C-5′ disclosed a pentose moiety in **1**. Further acid hydrolysis of **1** indicated the presence of D-xylose in **1** by thin-layer chromatography comparison with the authentic product and measurement of the specific rotation (xylose from **1**, $[\alpha]_D^{25} + 19°$; authentic D-xylose: $[\alpha]_D^{25} + 20°$). The *β* configuration of the sugar in **1** was determined by comparing the chemical shift due to the anomeric carbon with literature data (*α* configuration, 98–103 ppm; *β* configuration, 103–106 ppm) [19]. The linkage between cyathane diterpene unit and xylose moiety was confirmed by HMBC correlations from H-1′ to C-14.

The relative configuration of **1** was confirmed by analysis of the ROESY spectrum. The NOE correlations of H-14 with H_3-16 and H-10*α* indicated that H-14 and H_3-16 were on the same side (Figure S2). The NOE correlations of H-5 with H-10*β*, H-11, and H_3-17 placed them on the opposite face. The absolute configurations of C-5, C-6, C-9, C-11, and C-14 in **1** were assigned as 5*R*, 6*R*, 9*R*, 11*R*, and 14*S* by the similar negative Cotton effect around 210 nm and positive Cotton effect around 239 nm with those of **5** (Figure S3).

Erinacine U (**2**) possess a molecular formula of $C_{26}H_{40}O_7$, as determined by ESI HRMS ion at *m/z* 487.2667 $[M + Na]^+$ (calcd for $C_{26}H_{40}O_7Na$, 487.2666). The ^1H and ^{13}C NMR spectra of **2** (Table 1) were similar to those of **1**, except for an additional methoxyl group at δ_H 3.11 (3 H, s, H-21). The HMBC correlation from δ_H 3.11 to C-11 (δ_C 72.0) indicated **2** was a methylation product of **1** at the 11-OH. The *β* configurations of H-5, H-11, and H_3-17, and the *α* configuration of H-14 and H_3-16 were confirmed from the NOE cross peaks of H-5 with H-10*β*, H-11, and H_3-17, and H-16 with H-10*α* and H-14 (Figure S2). The identical negative Cotton effect at 212 nm and positive Cotton effect at 240 nm between **2** and **1** implied the same absolute
configuration of 5*R*, 6*R*, 9*R*, 11*R*, and 14*S* (Figure S3).

Erinacine V (**3**) has a molecular formula of $C_{26}H_{40}O_7$, as determined on the basis of the ESI HRMS ion at *m/z* 487.2669 $[M + H]^+$ (calcd for $C_{26}H_{40}O_7Na$, 487.2666). Interpretation of the ^1H, ^{13}C NMR and two-dimensional NMR spectra of **3** (Table 1) suggested the same planar structure as that of **2**. The NOE correlations of H-5 with H_3-17 and H-10*β*, H_3-16 with H-10*α* and H-14, and H-14 with H-11 (Figure S2) assigned the *β* configuration for H-5 and H_3-17 and the *α* configuration for H-11, H-14, and H_3-16 in **3**. Compound **3** is different from **2** in the stereochemistry of C-11.

Compounds **2** and **3** are *O*-methylated natural products. To confirm the origin of compounds **2** and **3**, the product of **1** under treatment with EtOAc and MeOH-water as used for the separation of **2** and **3** was analyzed by TLC. The fact that compound **1** was not transformed into **2** and **3** in the above test supports the natural origin of **2** and **3**. *O*-methylated cyathane diterpenes have been repeatedly isolated from the culture of *H. erinaceus*, which further supported that **2** and **3** are natural products.

The cytotoxicity and neurite-promoting activities of compounds **1**−**5** were evaluated using PC12 cell line. The cytotoxicities against PC12 cell line of **1**–**5** were tested by using CCK8 method. Only compound **4** showed weak cytotoxicity against PC12 cells with IC_{50} of 73.7 μM. The effects of compounds **1**–**5** on the neurite outgrowth of

Table 1 ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of **1–3** in DMSO-d_6

No.	**1**		**2**		**3**	
	δ_C	δ_H (mult. *J* in Hz)	δ_C	δ_H (mult. *J* in Hz)	δ_C	δ_H (mult. *J* in Hz)
1	37.9	1.55 m[a]	38.1	1.55 m	37.2	1.49 m
		1.48 m		1.47 m		
2	27.9	2.25 m	28.0	2.24 m	28.0	2.24 t (7.6)
3	138.1		138.4		138.2	
4	137.6		137.3		139.1	
5	39.5	1.83 m	39.5	1.85 m	35.6	3.06 o
6	44.0		43.6		44.6	
7	29.7	1.69 o[b]	29.6	1.75 m	31.4	1.91 o
		1.23 o		1.21 td (4.5, 13.1)		1.28 m
8	36.6	1.38 m	36.7	1.39 m	36.1	1.41 o
9	48.9		48.9		49.2	
10	32.2	2.30 o	29.1	2.39 dd (10.0, 13.6)	28.9	2.12 dd (3.9, 14.1)
		1.73 o		1.75 m		1.98 m
11	63.8	4.57 m	72.2	4.44 o	72.0	4.33 m
11-OH		5.05 d (5.9)				
11-OCH_3			55.8	3.12 s	55.7	3.11 s
12	140.6		137.5		141.7	
13	155.2	6.76 d (5.0)	158.8	6.99 d (5.4)	154.8	6,90 d (6.2)
14	84.1	4.44 d (5.0)	83.7	4.44 o	83.6	3.95 d (6.2)
15	194.1	9.42 s	193.7	9.49 s	194.5	9.41 s
16	15.8	0.93 s	15.6	0.93 s	16.5	0.80 s
17	24.6	0.90 s	24.5	0.89 s	24.3	1.02 s
18	26.4	2.82 m	26.5	2.82 m	26.5	2.93 m
19	21.3	0.95 d (7.0)	21.4	0.95 d (7.0)	22.0	0.95 d (7.5)
20	21.8	0.95 d (7.0)	21.7	0.95 d (7.0)	21.4	0.94 d (7.5)
1′	106.3	4.16 d (7.5)	106.2	4.12 d (7.5)	105.6	4.26 d (6.3)
2′	73.6	3.03 o	73.5	3.03 o	72.6	3.11 o
2′-OH		5.07 d (5.3)		5.08 d (5.1)		5.00 d (5.7)
3′	76.6	3.09 m	76.6	3.09 o	75.1	3.17 m
3′-OH		4.98 d (4.7)		5.00 d (4.3)		4.77 d (6.1)
4′	69.5	3.27 m	69.5	3.27 m	69.4	3.28 m
4′-OH		4.95 d (5.0)		4.95 d (4.8)		4.97 d (5.2)
5′	65.8	3.66 dd (5.3, 11.3)	65.8	3.66 dd (5.3, 11.2)	64.7	3.67 dd (4.6, 11.5)
		3.01 o		2.99 o		3.08 o

[a] "m" means signals multiplet

[b] "o" means overlapped with other signals

undifferentiated PC12 cells were evaluated by morphological observations and a quantitative analysis of neurite-bearing cells and neurite length. Compounds **1–3** and **5** showed significant neurotrophic effects in the range of 2.5–10 μM (Fig. 2, Figure S4−S6), as compared with control group. The percentage of neurite-bearing cells for cells treated with compounds **1–3** and **5** at 10 μM reached 43.7 ± 1.6%, 76.31 ± 1.7%, 65.3 ± 1.3%, and 48.3 ± 1.3%, respectively. NGF is used as positive control with neurite-bearing cells of 40.3 ± 0.7% at the concentration of 40 ng/mL.

In summary, three new compounds erinacines T–V (**1–3**) and two known compounds erinacine A (**4**) and erinacine P (**5**) were isolated from the liquid cultures of *H. erinaceus*. Compounds **1–3** and **5** exhibited significant neurite outgrowth-promoting effects on PC12 cells in the range of

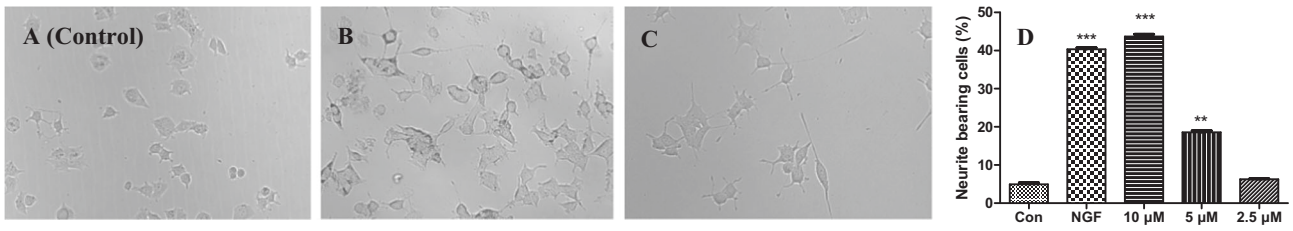

Fig. 2 Neurite outgrowth of PC12 cells after 24 h treatment with NGF and compound **1**, (**a**) Control, (**b**) NGF 40 ng/mL (**c**) compound **1** 10 μM. Cell with one or more neurites whose lengths were at least twice the diameter of the cell body were scored as positive. The percentage of positive neurite-bearing cells was determined from at least three different regions of interest in three independent experiments (**d**). Values were considered significant at $^{***}P < 0.001$, $^{**}P < 0.01$ versus control group

2.5–10 μM, providing novel leading compounds for neurotrophic agents to treat neurodegenerative diseases. Our research for these neurotrophic compounds further confirms the medicinal value of *H. erinaceus*.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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