Biosci. Biotechnol. Biochem., **64** (11), 2402–2405, 2000

Two Novel Diterpenoids, Erinacines H and I from the Mycelia of *Hericium erinaceum*

Eun Woo LEE,[1] Kazue SHIZUKI,[1] Satoshi HOSOKAWA,[1] Masami SUZUKI,[1] Hiroyuki SUGANUMA,[2] Takahiro INAKUMA,[2] Jingxuan LI,[3] Mayumi OHNISHI-KAMEYAMA,[4] Tadahiro NAGATA,[5] Shoei FURUKAWA,[6] and Hirokazu KAWAGISHI[1,†]

[1]*Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Shizuoka 422-8529, Japan*
[2]*Research Institute, Kagome Co., Ltd., 17 Nishitomiyama, Nishinasuno-machi, Tochigi 329-2762, Japan*
[3]*Jilin Institute of Biology, 6 Qian, Jin Street, Changchun, China*
[4]*National Food Research Institute, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki 305-8642, Japan*
[5]*Department of Animal Products, National Institute of Animal Industry-NIAI, 2 Ikenodai, Kukizaki, Ibaraki 305-0901, Japan*
[6]*Department of Molecular Biology, Gifu Pharmaceutical University, 5-6-1 Mitahora-Higashi, Gifu 502-8585, Japan*

Revised May 19, 2000; Accepted June 27, 2000

Novel diterpenoids, erinacines H (1) and I (3), were isolated from the cultured mycelia of *Hericium erinaceum*. The structures of the compounds were determined by interpretation of the spectral data. Erinacine H showed stimulating activity of nerve growth factor (NGF)-synthesis.

Key words: *Hericium erinaceum*; nerve growth factor; stimulator; diterpenoid

Stimulators of nerve growth factor (NGF) are candidates for medicines for degenerative neuronal disorders and peripheral nerve regeneration. Some natural products with such activity have been reported.[1-10] In previous papers, we reported the isolation of such stimulators, hericenones C to H, from the fruiting bodies of *Hericium erinaceum* and erinacines A to G from the mycelia of the fungus.[1-5] In this paper, we describe isolation of new compounds, erinacines H and I, from the mycelia.

Materials and Methods

General procedures. Optical rotations were measured on a JASCO DIP-1000 desital polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL lambda-500 spectrometer. The IR spectra were recorded on a JASCO A-102 diffraction grating infrared spectrometer. FABMS and HRFABMS spectra were recorded on a JEOL DX-303HF mass spectrometer.

Extraction and isolation. H. erinaceum was cultivated by shaking at 30°C for 4 weeks. The culture was centrifuged, and the precipitate (mycelia, wet weight; 1.50 kg) were extracted with ethanol. The extract, after concentrating the solvent, was fractionated by solvent partition between ethyl acetate and water. The ethyl acetate-extract (40.2 g) was separated by column chromatography on silica gel using $CHCl_3$-acetone and then $CHCl_3$-MeOH mixtures of increasing polarity, to give 11 fractions.

Fraction 3 was chromatographed using Toyopearl HW-40 (MeOH), giving 9 fractions (Fraction 3-1 to 3-9). Erinacine I (10.3 mg) was isolated from Fraction 3-5 by preparative TLC ($CHCl_3$-MeOH, 98:2) and then HPLC (ODS, MeOH-H_2O, 9:1).

Fraction 8 was chromatographed using silica gel ($CHCl_3$-MeOH, 85:15), giving 11 fractions (Fraction 8-1 to 8-11). Erinacine H (6.0 mg) was isolated from Fraction 8-5 by preparative TLC ($CHCl_3$-MeOH, 85:15) and then HPLC (ODS, MeOH-H_2O, 8:2).

Erinacine H (1): yellowish amorphous residue; $[\alpha]_D$ +168° (*c* 0.480, MeOH). IR (KBr) ν_{max} 3431, 1637, 1560 cm^{-1}. ^{1}H and ^{13}C NMR, see Table 1. FABMS (positive; matrix, 3-nitrobenzyl alcohol)

† To whom correspondence should be addressed. Hirokazu KAWAGISHI, Tel and Fax: +81-54-238-4885; E-mail: achkawa@agr.shizuoka.ac.jp

Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1–3**[a]

Position	Compound					
	1 (CD_3OD)		**2** ($CDCl_3$)		**3** ($CDCl_3$)	
1	1.72 (m)	40.7	1.67 (m)	38.2	1.62 (m)	39.9
	1.67 (m)		1.57 (m)		1.41 (m)	
2	2.36 (dd, 8.9, 5.9)	31.6	2.34 (m)	28.8	2.27 (m)	28.7
3		144.8		145.4	2.21 (m)	139.5
4		143.5		141.6		136.1
5		139.4		138.5	2.36 (m)	39.5
6		n.d[b]		47.9		42.1
7	2.26 (m)	33.9	2.36 (m)	33.2	1.51 (m)	30.4
	1.51 (br.d, 15.8)		1.30 (br.d, 13.2)		1.30 (m)	
8	1.63 (m)	38.8	1.61 (m)	36.3	1.51 (m)	37.0
9		n.d		49.1		48.2
10	5.60 (d, 7.9)	122.9	5.81 (d, 8.1)	119.8	2.21 (m)	26.7
					1.51 (m)	
11	6.77 (d, 7.9)	130.9	6.72 (d, 8.1)	145.4	4.71 (m)	79.6
12		148.3		154.0		143.1
13	3.01 (m)	34.8	3.24 (dd, 17.6, 5.9)	27.5	6.07 (s)	129.1
			2.48 (d, 17.6)			
14	3.55 (d, 5.9)	88.7	3.60 (d, 5.9)	84.0		110.2
15		177.9	9.31 (s)	194.2	4.77 (d, 13.9)	59.8
					4.66 (d, 13.9)	
16	0.98 (s)	28.7	0.93 (s)	26.3	1.01 (s)	11.4
17	1.04 (s)	24.9	0.93 (s)	23.8	0.93 (s)	23.9
18	2.86 (heptet, 6.9)	30.1	2.77 (heptet, 6.6)	26.8	2.89 (heptet, 6.7)	26.3
19, 20	1.00 (d, 6.9)	22.6	0.98 (d, 6.6)	21.4	0.98 (s)	22.3
	0.96 (d, 6.9)	22.5	0.91 (d, 6.6)	21.4	0.89 (s)	21.3
1′	4.36 (d, 6.6)	107.7	4.48 (d, 5.1)	104.8		
2′	3.25 (dd, 6.6, 6.6)	75.2	3.38 (dd, 5.1, 6.6)	71.5		
3′	3.41 (m)	77.6	3.46 (dd, 6.6, 7.0)	73.2		
4′	3.43 (m)	72.0	3.50 (m)	69.3		
5′	3.88 (dd, 11.6, 4.6)	66.9	3.74 (dd, 11.7, 2.9)	63.5		
	3.19 (m)		3.20 (dd, 11.7, 7.0)			
Ac					2.01 (s)	20.7
AcCO						170.6

[a] Assignments were established by DEPT, HSQC, HMBC, COSY and NOESY analysis.
[b] Not measured because these signals were overlapped with the solvent signals.

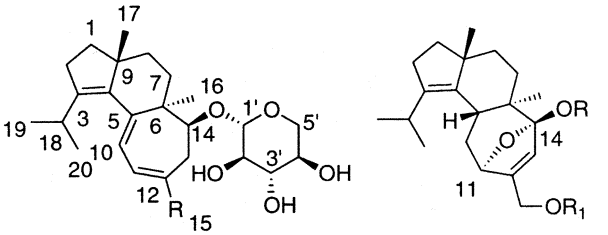
m/z 471 $[M+H]^+$, 493 $[M+Na]^+$. HRFABMS *m/z* $[M+H]^+$: Calcd for $C_{25}H_{35}O_7Na$: 471.2359, Found: 471.2350.

Erinacine I (3): yellowish oil; $[\alpha]_D -110°$ (*c* 0.98, MeOH). IR (film) ν_{max} 3391, 1745 cm^{-1}. ^{1}H and ^{13}C NMR, see Table 1. FABMS (positive; matrix, 3-nitrobenzyl alcohol) *m/z* 383 $[M+Na]^+$. HRFABMS *m/z* $[M+Na]^+$: Calcd for $C_{22}H_{32}O_4Na$: 383.2198, Found: 383.2199.

NGF stimulating assay. Quiescent astroglial cells from rats were used for the assay. To the cells maintained in wells of a 96-well microplate, compound **1** at various concentrations was added. The culture was kept for 24 h and then NGF secreted into the culture medium was measured by an enzyme immunoassay.[1-5,11,12-15]

Results and Discussion

Cultured *H. erinaceum* was extracted with ethanol and the extract was fractionated by solvent partition between ethyl acetate and water. Repeated column chromatography, TLC, and HPLC of the ethyl acetate-extract gave two new compounds.

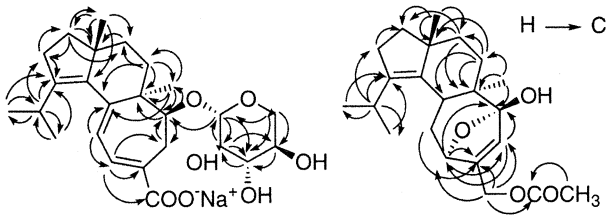


1: R = COO^-Na^+
2: R = CHO

3: R = H R_1 = Ac
4: R = CH_3 R_1 = Ac
5: R = CH_3 R_1 = H
6: R = H R_1 = H

Structure 1

The molecular formula $C_{25}H_{35}O_7Na$ of erinacine H (**1**) was determined by HRFABMS of the $[M+H]^+$ ion (471.2350, calcd. 471.2359). The ^{13}C and ^{1}H NMR data were similar to those of erinacine A (**2**)



1 3

Fig. 1. HMBC Correlations of Erinacines H (**1**) and I (**3**).

(Table 1); both being diterpenoids with a "cyathane" skeleton and a xylose moiety.[3,16,17] The sugar part of **1** was confirmed by HCl hydrolysis of **1** followed by HPLC analysis of the resulting monosaccharide (data not shown). Compound **1** differs from erinacine A (**2**) by the presence of a carboxyl group (δ 177.9 in ^{13}C NMR) instead of a formyl group of **2**. The complete planar structure was determined by interpretation of HMBC correlations (Fig. 1). The relative stereochemistry was determined by NOESY experiment; the NOE appeared between H14/H16, H7a (δ 1.51)/H16, and H7b (δ 2.26)/H-17. The absolute configuration of **1** was deduced by comparison of its $[\alpha]_D + 168°$ (*c* 0.480, MeOH) with that [$+216°$ (*c* 0.280, MeOH)] of **2** whose absolute configuration is known.[3]

Erinacine I (**3**) has the molecular formula $C_{22}H_{32}O_4$ as found by HRFABMS of the $[M+Na]^+$ ion (383.2199, calcd. 383.2198). The structure was mainly determined by HMBC experiment (Fig. 1). This structure could exist as the keto-ketol tautomeric pair at C14. In fact, this compound always gave two spots on TLC. However, the keto form of the compound was not observed under NMR measuring conditions. The relative stereochemistry was partially determined on the basis of NOESY data. Observed cross peaks are as follows; H5/H17, H5/H10a (δ 1.51), H16/H10b(δ 2.21). The stereochemistry at C11 and C14 could not be clarified by the NOESY experiments. However, the relative stereochemistry probably is as **3**, because its ^1H and ^{13}C NMR data were very similar with those of **4** which was isolated from the bird's nest fungus *Cyathus helenae*.[16,17] The absolute configuration of **3** was deduced by comparison of its optical rotation with those of **5** and **6** whose absolute configurations have been determined: **3**, $[\alpha]_D - 110°$ (*c* 0.98, MeOH); **5**, $-154°$ (*c* 0.24, MeOH); **6**, $-155°$ (*c* 0.26, MeOH).[17]

Erinacine H (**1**) showed stimulating activity to the synthesis of NGF using astroglial cells. The amounts (31.5 ± 1.7 pg/ml) of NGF secreted into the medium in the presence of 33.3 μg/ml of **1** was five times greater than those in the absence of the compound.[1-5,11,12-15]

References

1) Kawagishi, H., Ando, M., Sakamoto, H., Yoshida, S., Ojima, F., Ishiguro, Y., Ukai, N., and Furukawa, S., Hericenones C, D, and E, stimulators of nerve growth factor (NGF)-synthesis, from the mushroom *Hericium erinaceum*. *Tetrahedron Lett.*, **32**, 4561–4564 (1991).
2) Kawagishi, H., Ando, M., Shinba, K., Sakamoto, H., Yoshida, S., Ishiguro, Y., and Furukawa, S., Chromans, hericenones F, G, and H, from the mushroom *Hericium erinaceum*. *Phytochemistry*, **32**, 175–178 (1993).
3) Kawagishi, H., Shimada, A., Shirai, R., Okamoto, K., Ojima, F., Sakamoto, H., Ishiguro, Y., and Furukawa, S., Erinacines A, B, and C, strong stimulators of nerve growth factor (NGF)-synthesis, from the mycelia of *Hericium erinaceum*. *Tetrahedron Lett.*, **35**, 1569–1572 (1994).
4) Kawagishi, H., Shimada, A., Shizuki, K., Mori, H., Okamoto, K., Sakamoto, H., and Furukawa, S., Erinacine D, a stimulator of NGF-synthesis, from the mycelia of *Hericium erinaceum*. *Heterocycl. Commun.*, **2**, 51–54 (1996).
5) Kawagishi, H., Shimada, A., Hosokawa, S., Mori, H., Sakamoto, H., Ishiguro, Y., Sakemi, S., Bordner, J., Kojima, N., and Furukawa, S., Erinacines E, F, and G, stimulators of nerve growth factor (NGF)-synthesis, from the mycelia of *Hericium erinaceum*. *Tetrahedron Lett.*, **37**, 7399–7402 (1996).
6) Kawagishi, H., Ishiyama, D., Mori, H., Sakamoto, H., Ishiguro, Y., Furukawa, S., and Li, J., Dictyophorines A and B, novel stimulators of NGF-synthesis, from the mushroom *Dictyophora indusiata*. *Phytochemistry*, **45**, 1203–1205 (1997).
7) Kita, T., Takaya, Y., Oshima, Y., Ohta, T., Aizawa, K., Hirano, T., and Inakuma, T., Scabronines B, C, D, E, and F, novel diterpenoids showing stimulating activity of nerve growth factor-synthesis, from the mushroom *Sarcodon scabrosus*. *Tetrahedron*, **54**, 11877–11886 (1998).
8) Yamaguchi, K., Tsuji, T., Wakui, S., Yazawa, K., Kondo, K., Shigemori, H., and Kobayashi, J., Stimulation of nerve growth factor synthesis and secretion by fellutamide A *in vitro*. *Biosci. Biotechnol. Biochem.*, **57**, 195–199 (1993).
9) Yamaguchi, K., Sasano, A., Urakami, T., Tsuji, T., and Kondo, K., Stimulation of nerve growth factor production by pyrroloquinolinequinone and its derivatives *in vitro* and *in vivo*. *Biosci. Biotechnol. Biochem.*, **57**, 1231–1233 (1993).
10) Yamaguchi, K., Uemura, D., Tsuji, T., and Kondo, K., Stimulation of nerve growth factor production by diterpenoids isolated from plants of *Euphorbia* species. *Biosci. Biotechnol. Biochem.*, **58**, 1749–1751 (1994).
11) Furukawa, Y., Furukawa, S., Satoyoshi, E., and Hayashi, K., Catecholamines induce an increase in nerve growth factor content in the medium of mouse L-M cells. *J. Biol. Chem.*, **261**, 6039–6047 (1986).
12) Shinoda, I., Furukawa, Y., and Furukawa, S., Stimulation of nerve growth factor synthesis/secre-

tion by propentofylline in cultured mouse astroglial cells. *Biochem. Pharm.*, **39**, 1813 (1990).
13) Furukawa, Y., Furukawa, S., Ikeda, F., Satoyoshi, E., and Hayashi, K., Aliphatic side chain of catecholamine potentiates the stimulatory effect of the catechol part on the synthesis of nerve growth factor. *FEBS Lett.*, **208**, 258–262 (1986).
14) Furukawa, S., Furukawa, Y., Satoyoshi, E., and Hayashi, K., Synthesis and secretion of nerve growth factor by mouse astroglial cells in culture. *Biochem. Biophys. Res. Commun.*, **136**, 57–63 (1986).
15) Furukawa, S., Furukawa, Y., Satoyoshi, E., and Hayashi, K., Regulation of nerve growth factor synthesis/secretion by catecholamine in cultured mouse astroglial cells. *Biochem. Biophys. Res. Commun.*, **147**, 1048–1054 (1987).
16) Ayer, W. A., Nakashima, T. T., and Ward, D. E., Metabolites of bird's nest fungi. Part 10. Carbon-13 nuclear magnetic resonance studies on the cyathins. *Can. J. Chem.*, **56**, 2197–2199 (1978).
17) Ayer, W. A. and Taube, H., Metabolites of *Cyathus helenae*. A new class of diterpenoids. *Can. J. Chem.*, **51**, 3842–3853 (1973).