Short Communication

Hericerin, a New Pollen Growth Inhibitor from the Mushroom *Hericium erinaceum*

Yasuo KIMURA, Masahiko NISHIBE,
Hiromitsu NAKAJIMA, Takashi HAMASAKI,
Atsumi SHIMADA, Akihiko TSUNEDA*
and Norihiro SHIGEMATSU**

Department of Bioresource Science,
Faculty of Agriculture, Tottori University,
Koyama, Tottori 680, Japan

*The Tottori Mycological Institute,
Kokoge, Tottori 689-11, Japan

**Daiwabo Create Co., Ltd.,
Komiya, Harima-cho, Kakogun,
Hyogo 675-01, Japan

Received April 22, 1991

Papers¹⁻³⁾ on the biologically active substances contained in the mushroom, *Hericium erinaceum* (Yamabushitake in Japanese), have been published. During the course of our screening program for pollen growth regulators among the extracts of mushrooms, it was found that one mushroom, *Hericium erinaceum*, contained a new biologically active substance which we have named hericerin (**1**). In this paper, we describe the isolation and structural determination of **1** together with an inhibitory effect on pine pollen germination and tea pollen growth.

The mushroom (500 g fresh weight), which had been artificially cultured, was extracted three times with acetone. After evaporating the solvent, the remaining aqueous solution was partitioned with EtOAc at pH 4.5 and the organic layer was concentrated *in vacuo*. The residue was purified by column

chromatography (benzene–EtOAc, 9:1, v/v) on silica gel and then by TLC (benzene–EtOH, 8:2, v/v) on Kieselgel. The active principle thus obtained was recrystallized from benzene–EtOAc to afford pure colorless needles, mp 138–140°C in a yield of 25 mg.

The molecular formula was determined by HR-EIMS (obs. m/z 419.2425, M^+) to be $C_{27}H_{33}NO_3$ and confirmed by elementary analysis (Found: C, 75.82; H, 7.80; N, 3.28. Calcd: +1/2 H_2O ; C, 75.70; H, 7.94; N, 3.27%), ^{13}C -NMR and 1H -NMR, implying 12 degrees of unsaturation. Homo-spin decoupling experiments in the 1H -NMR spectrum and the ^{13}C chemical shifts showed the presence of a (2'*E*)—geranyl group.^{4,5)} 1H spin-decoupling experiments as well as the mass spectral analysis indicated the presence of a phenylethyl moiety (m/z , 105) as a partial structure of **1**. Since the signal at δ_H 3.85 (2H, m) assignable to 1'' methylene appeared at δ_C 44.2 in the ^{13}C -NMR spectrum, this was coupled to the adjacent benzylic protons at δ_H 2.97 (2H, t). A tertiary nitrogen atom which did not have D_2O exchangeable protons seemed to be adjacent to the 1'' carbon from the chemical shift.

The signals at δ_H 3.84 (3H, s) and δ_C 56.1 (s), and D_2O exchangeable resonance at δ_H 6.19 (1H, s) were assigned to a methoxyl group and a phenolic hydroxyl group, respectively. The signal at δ_H 6.95 (1H, s) was assigned to an aromatic proton. From the molecular formula of **1**, the remaining atoms were assigned to $-CO-$ (δ_C 168.9) and $-CH_2-$ [δ_H 4.16 (2H, s)] groups attached to a nitrogen atom by the chemical shifts. These data together with the IR spectrum (1660 cm^{-1}) indicated the presence of a isoindolinone nucleus in **1**.

In DIFNOE experiments, NOE effects were observed between the signals at δ_H 6.95 and δ_H

Fig. 1.

Fig. 2.

Fig. 3.

Table I. ^1H - AND ^{13}C -NMR ASSIGNMENTS FOR HERICERIN

No.	δ_{C}	δ_{H}
1	168.9 s	
2		
3	48.1 t	4.16 2H, s
3a	132.1 s	
4	97.7 d	6.95 1H, s
5	150.5 s	
6	118.4 s	
7	158.4 s	
7a	121.1 s	
1'	22.8 t	3.49 2H, d, $J=6.8$
2'	121.2 d	5.24 1H, t, $J=6.8$
3'	138.7 s	
4'	39.7 t	2.08 2H, s
5'	26.3 t	2.08 2H, s
6'	123.6 d	5.03 1H, br, s
7'	132.2 s	
8'	17.1 q	1.58 3H, s
9'	16.2 q	1.81 3H, s
10'	25.7 q	1.66 3H, s
1''	44.2 t	3.85 2H, m
2''	34.9 t	2.97 2H, t, $J=7.3$
3''	139.5 s	
4'', 8''	128.7 d	7.24
5'', 7''	128.6 d	7.24 > 5H, m.
6''	126.5 d	7.24
OH		6.19 1H, s
OCH ₃	56.1 q	3.84 3H, s

 ^{13}C at 67.80 MHz; ^1H at 270.05 MHz.

4.16, and at δ_{H} 6.95 and δ_{H} 3.84. The position of the remaining groups, a hydroxyl group and a geranyl group, was established by the LSPD experiments to be as shown in Fig. 2, a methoxyl group (δ_{H} 3.84) being attached to a carbon at δ_{C} 150.5. By irradiating the signal at δ_{H} 3.49 due to the terminal methylene group of a geranyl group bound to the benzene ring, the carbons at δ_{C} 150.5 and 158.4 were changed.

These results indicate that a hydroxyl group was in a *meta*-position to a methoxyl group.

All the data for ^1H and ^{13}C -NMR were determined by selective proton decoupling and LSPD experiments and compared with literature values.^{4,5)} Thus, the structure of **1** was established to be 6-[(2'*E*)-3',7'-dimethyl-2',6'-octadienyl]-7-hydroxy-5-methoxy-*N*-(2''-phenylethyl)-1-isoinдолinone.

Hericerin showed inhibitory activity against pine pollen germination⁶⁾ and tea pollen growth.⁷⁾ As shown in Fig. 3, **1** at 30 mg/l inhibited germination by about 25% over the control, and completely inhibited it at a concentration of 100 mg/l. On the other side, **1** exhibited growth inhibitory activity of about 65% and 80% at concentrations of 10 mg/l and 30 mg/l against tea pollen, respectively. Tea pollen growth was markedly inhibited at the 100 mg/l.

Acknowledgment. The authors thank Dr. F. Sugawara of The Institute of Physical and Chemical Research for measuring the mass spectra.

References

- 1) Xie Fei-Jun, Su Yi-Hua, Xu Ji-Ping and Huang Da-Xian, *Pharma Medica*, **1986**, 139.
- 2) H. Kawagishi, M. Ando and T. Mizuno, *Tetrahedron Lett.*, **31**, 373 (1990).
- 3) H. Kawagishi, M. Ando, T. Mizuno, H. Yokota and S. Konishi, *Agric. Biol. Chem.*, **54**, 1329 (1990).
- 4) M. Jautelat, J. B. Grutzner and J. D. Roberts, *Proc. Natl. Acad. Sci. U.S.A.*, **65**, 288 (1970).
- 5) C. Nishino and S. Bowers, *Tetrahedron*, **32**, 2875 (1976).
- 6) Y. Kimura, A. Shimada, H. Nakajima and T. Hamasaki, *Agric. Biol. Chem.*, **52**, 1253 (1988).
- 7) S. Konishi and H. Yokota, *Plant & Cell Physiol.*, **21**, 255 (1980).