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HERICENONE A AND B AS CYTOTOXIC PRINCIPLES FROM THE MUSHROOM HERICIUM ERINACEUM

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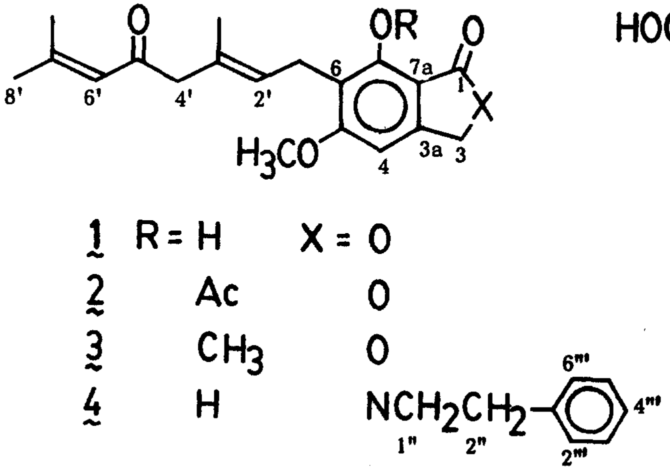
Abstract: Novel cytotoxic phenols, hericenone A (1) and B (4) were isolated from the mushroom Hericium erinaceum. These structures were determined by interpretation of spectral data and chemical analyses.

In the course of our continuing research aimed at the isolation of biological active compounds from mushrooms[1), we found the two cytotoxic phenols 1 and 4 in a edible mushroom Hericium erinaceum which were cultured in Japan. These compounds showed cytotoxicity against HeLa cells.

Fresh fruiting bodies of H. erinaceum (7.3 kg) were extracted with acetone, and the extract was concentrated and fractionated by solvent partitions (chloroform and then ethyl acetate). Repeated column chromatography (SiO_2) followed by recrystalization of the chloroform extract, which exhibited more potent activity than the ethyl acetate one, gave 1 (3.0 mg, mp 100-102℃) and 4 (3.2 mg, mp 136-138℃) as colorless crystals.



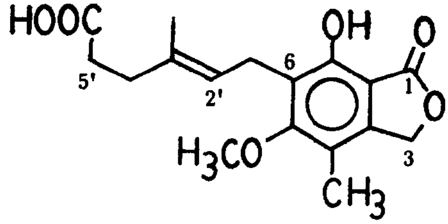


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Table ^{1}H NMR assignments for compounds (1) ~ (4)*

	ppm(multiplicity, J in Hz) in $CDCl_3$ 1	2	3	4
H-3	5.25(s)	5.12(s)	5.40(s)	4.20(s)
4	6.97(s)	7.25(s)	7.10(s)	6.96(s)
1'	3.59(d,6.41)	3.43(d,6.60)	3.49(d,6.59)	3.56(d,6.74)
2'	5.30(t,6.41)	5.17(t,6.60)	5.26(t,6.59)	5.30(t,6.74)
4'	3.18(s)	3.03(s)	3.03(s)	3.14(s)
6'	6.09(s)	6.05(s)	6.07(s)	6.08(s)
8'	1.91(s)	1.85(s)	1.85(s)	1.88(s)
3'-CH_3	1.81(s)	1.77(s)	1.79(s)	1.81(s)
7'-CH_3	2.17(s)	2.13(s)	2.13(s)	2.16(s)
1"				3.84(t,7.33)
2"				2.97(t,7.33)
2''' -6'''				7.20-7.26(m)
OCH_3	3.89(s)	3.90(s)	3.88(s) 3.90(s)	3.84(s)
Ac		2.35(s)		

*These assignments were established by the decoupling, HH-, CH-COSY, NOE difference, and/or NOESY analyses.

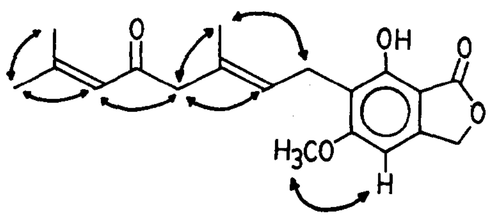

Fig. NOEs (←→) of 1 in the NOE diffrense and/or NOESY experiments.

FAB-MS of 1 exhibited MH^+ ion at m/z 331. The molecular formula $C_{19}H_{22}O_5$ was assigned by HR-EI-MS of of the M^+ ion (330.1494 Δ+2.7 mmu)[2). The ^{1}H (Table) and ^{13}C NMR[3)] data are similar to those of mycophenolic acid[4)] (5) and suggested a methylene, a penta-substituted phenyl, a methoxy, a hydroxy, two carbonyl groups, and a C10 side chain including two olefins. The two carbonyl groups (171.86 and 199.08 ppm in ^{13}C NMR spectrum) were assigned to a phthalide (1760 cm^{-1}) and an α,β-unsaturated ketone (1660 cm^{-1}) from the IR data. The IR spectrum also indicates the presence of a hydrogen-bonded hydroxy group (3300-2600 cm^{-1}), which reacted with Folin Ciocalteu reagent, suggesting this group is a phenol at ortho-position of the phthalide-carboxyl group and form hydrogen bond to the group. The carbon appeared at 98.45 ppm in ^{13}C NMR spectrum was assigned to an unsubstituted one of the benzene ring on the basis of C-H COSY data, and its higher field shift can be explained in the terms of the presence of hydroxy and methoxy groups at both ortho-, or ortho and para-carbons of the unsubstituted one. In addition, the NOEs appeared between the phenyl and methoxy protons in the NOE difference and NOESY spectra, suggesting vicinity of the phenyl proton and the methoxy group (Fig.). Furthermore, 1 gave mono-acetate 2[5] (Table) with acetic anhydride and pyridine, and methyl ether 3[6] (Table) with K_2CO_3 and CH_3I. In the NOE difference experiments of the ether 3, no NOE between phenyl and newly induced-methoxy protons could be observed and the NOEs appeared at both methoxy groups by irradiation at H-1' methylene protons, indicating that C10 chain is attached at ortho-position of both of methoxy and hydroxy groups. ^1H NMR of 1 are less informative since most of the signals appeared as singlets (Table); the structure of C10 chain was determined mainly by analyses of the NOE difference and NOESY spectra (Fig.). All the results allow us to conclude that the structure of hericenone A is 6-[(2'E)-3',7'-dimethyl-5'-oxo-2',6'-octadienyl]-7-hydroxy-5-methoxyphthalide (1).

Compound 4 showed MH^+ ion at m/z 434 and $M+Na^+$ ion at 456 in FAB-MS, and has the molecular formula $C_{27}H_{31}NO_4$ from HR-EI-MS of M^+ ion (m/z 433.2243 Δ-1.0 mmu)[7]. The ^1H NMR data (Table) are similar to those of 1 except for H-3, additional two methylene and phenyl protons; H-3 of 4 appeared at 4.20 (s) while that of 1 appeared at δ 5.25 (s), and 4 has two methylene protons at δ 2.97 (H-1", t) and 3.84 (H-2", t) which are coupled each other (J=7.22 Hz), and a mono-substituted phenyl group at 7.20-7.26(m). The IR spectrum shows that 4 has γ-lactam (1680 cm-1) instead of γ-lactone in 1. In addition, the NOE was observed between H-3 and H-1" in the NOE difference experiments. All the data are in full agreement with the proposed structure; 6-[(2'E)-3',7'-dimethyl-5'-oxo-2',6'-octadienyl]-7-hydroxy-5-methoxy-N-(2"-phenylethyl)-1-isoindolinone (4)[8].

The minimum concentrations giving complete growth inhibition of HeLa cells for hericenone A (1) was 100 μg/ml, for hericenone B (4) was 6.3 μg/ml; the potent cytotoxicity of 4 may be due to γ-lactam and its N-substituent.

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References and notes

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2) EI-MS of 1 (JEOL DX-302 mass spectrometer), m/z (rel. int. %): 330[M^+](21.0), 312(13.2), 284(3.9), 193(9.2), 137(17.0), 97(11.7), 83(100.0), 55(69.8)

3) ^{13}C NMR of 1 (JEOL GSX-400 spectrometer): 199.08(C-5'), 171.86(C-1), 159.18(C-5 or 7), 157.48(C-7 or 5), 150.61(C-7'), 133.63(C-3a), 128.22(C-3'), 125.80(C-7a or 2'), 125.02(C-2' or 7a), 123.05(C-6'), 121.35(C-6), 98.45(C-4), 68.31(C-3), 56.21 (OCH_3), 54.40(C-4'), 27.82(C-8'), 23.32(C-1'), 21.06(C-7'-CH_3), 17.18(C-3'-CH_3)

4) Isolation: B. Gosio, Riv. Igriene Sanita publica Ann., 7, 825 (1896). Structure determination: J. H. Birkinshaw, H. Raistrick and D. J. Ross, Biochem. J., 50, 630 (1952); W. R. Logan and G. T. Newbold, J. Chem. Soc., 1946 (1957). Biological activity: K. Ando, S. Suzuki, G. Tamura and K. Arima, J. Antibiot. 21, 649 (1968); S. B. Carter, T. J. Franklin, D. F. Jones, B. J. Leonard, S. D. Mills, R. W. Turner and W. B. Turner, Nature(London), 223, 848 (1969); T. J. Franklin and J. M. Cook, Biochem. J., 113, 515 (1969); M. J. Sweeney, K. Gerzon, P. N. Harris, R. E. Holmes, G. A. Poore and R. H. Williams, Cancer. Res., 32, 1795 (1972); M. J. Sweeney, D. H. Hoffman and M. A. Esterman, Cancer Res., 32, 1803 (1972). Synthesis: A. J. Birch and J. J. Wright, Aust. J. Chem., 22, 2635 (1969); L. Canonica, B. Rindone, E. Santaniello and C. Scolastico, Tetrahedron, 28, 4395 (1972); R. L. Danheiser, S. K. Gee and J. J. Perez, J. Am. Chem. Soc., 108, 806 (1986). Biosynthesis: L. Canonica, W. Kroszczynski, B. M. Ranzi, B. Rindone, E. Santaniello and C. Scolastico, J. Chem. Soc. Perkin I, 2639 (1972). ^{1}H NMR of 5: 1.90(H-3',s), 2.15(4-CH_3,s), 2.28-2.34(H-4'or 5',m), 2.41-2.48(H-5'or 4',m), 3.39(H-1',d,6.9), 3.76(OCH_3,s), 5.20(H-3,s), 5.20-5.30(H-2',m); ^{13}C NMR of 5: 11.4, 16.0, 22.5, 32.6, 34.1, 60.9, 70.0, 106.2, 116.6, 122.0, 122.8, 133.8,144.0, 153.5, 163.5, 172.8, 179.1 (from Danheiser et al., 1986)

5) EI-MS of 2, m/z(rel. int. %): 372[M^+](11.5), 312(1.8), 290(2.1), 247(4.4), 193(3.9), 137(7.6), 83(100.0), 55(40.0)

6) EI-MS of 3, m/z(rel. int. %); 344[M^+](13.7), 256(11.1), 207(7.2), 137(12.0), 97(14.6), 83(100.0), 69(73.9), 55(54.0)

7) EI-MS of 4, m/z(rel. int. %); 433[M^+](12.9), 342(64.6), 205(3.3), 137(4.5), 105(15.5), 83(100.0), 55(36.3)

8) ^{13}C NMR of 4: 198.98(C-5'), 168.94(C-1), 158.48(C-5 or 7), 156.81(C-7 or 5), 150.70(C-7'), 138.76(C-1'''), 132.85(C-3a or 4'''), 132.20(C-4''' or 3a), 128.70 (C-3'), 128.61(C-2''',-6'''), 126.51(C-2'), 123.01(C-6'), 122.00(C-7a), 118.48 (C-6), 97.69(C-4), 56.21(OCH_3), 54.65(C-4'), 48.28(C-3), 44.21(C-1''), 34.90 (C-2''), 27.76(C-8'), 23.09(C-1'), 20,94(C-7'-CH_3), 17.00(C-3'-CH_3)

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