カイメンタケ（*Phaeolus schweinitzii*）の抗酸化成分

Isolation of Antioxidative Compounds from *Phaeolus schweinitzii*

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Abstract

The antioxidative activity and active compounds of the fruit bodies of the mushroom, *Phaeolus schweinitzii* were studied. The ethanol extract of *Phaeolus schweinitzii* showed strong antioxidative activity. Its activity being comparable to that of *t*-butylhydroxyanisol (BHA).

Chromatographic purification of this extract gave an active compound identified as hispidin. Hispidin showed higher antioxidative activity than α–tocopherol and BHA.

Keywords : mushroom, *Phaeolus schweinitzii*, antioxidant, radical-scavenging activity, hispidin

In our study to find new antioxidants from the Basidiomycetes, we reported several species of mushrooms which showed a significant antioxidative activity against methyl linoleate[1, 2] .

In this paper, we wish to report the isolation and characterization of the active principle of *Phaeolus schweinitzii* (Fig. 1) .

The ethanol extract of *P. schweinitzii* was concentrated *in vacuo* to yield a residue which was partitioned between EtOAc and H_2O. The EtOAc layer was concentrated to give a residue which was chromatographed on Sephadex LH-20（$CHCl_3$-MeOH=1:1）to give the antioxidant 1 as a brown needles (2.344g) (Fig.2).

The molecular formula of 1 was determined to be $C_{13}H_{10}O_5$ by HRMS. Analysis of the DEPT and HMBC spectra of 1 in conjunction with the ^{1}H- and ^{13}C- NMR spectra indicated that the chemical structure of 1 was 4-hydroxy-6- (3, 4-dihydroxystyryl) pyrone (Fig. 3).

Compound 1 has been isolated from *Polyporus hispidus* as hispidin[3]. However, there has been no previous report on antioxidative activity of 1.

The antioxidative activity of 1 was assayed by measurement of radical-scavenging activity [4]. A comparison with *t*-butyl hydroxyanisol (BHA) and α-tocopherol indicated that the activity of hispidin was stronger than that of two standard antioxidants, and was 1.7 times stronger than α-tocopherol (Fig. 4).

Fig.1. *Phaeolus schweinitzii*

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Experimental

Apparatus

Melting point was measured on the microscope hot plate of a Yanagimoto MP-J3 instrument.

IR spectra were recorded on a Shimadzu IR-408 infrared spectrometer, and ^{1}H (600MHz) and ^{13}C (150MHz) NMR spectra were obtained with a Varian-Unity 600 spectrometer, using tetramethylsilane as an internal standard. Chemical shift data were recorded as δ values. High- and low- resolution mass spectra were measured by a JEOLJMS-AX-500 spectrometer, and UV spectra were recorded on a Shimadzu UV-300 spectrometer.

Extraction and isolation

The fresh fruit bodies of *Phaeolus schweinitzii* collected in Nagano, Japan were dried at 60℃ and ground mechanically to give powder (155g) which was extracted 2 times with EtOH (2 liter) at room temperature for 2 weeks.

The EtOH extract was concentrated to yield a residue (15.8g).

The residue was partitioned between EtOAc and H_2O, and the EtOAc layer was concentrated to give residue (9.1g) which was done a chromatographed on a Sephadex LH-20 (20φ × 400mm) using a mixed solvent system of CHCl3-MeOH (1:1, v/v) to give Fr.1～11. The Fr.9~11 were combined and concentrated to give hispidin 1 as brown needles (2.344g), mp. 239 ~ 241° (dec.).

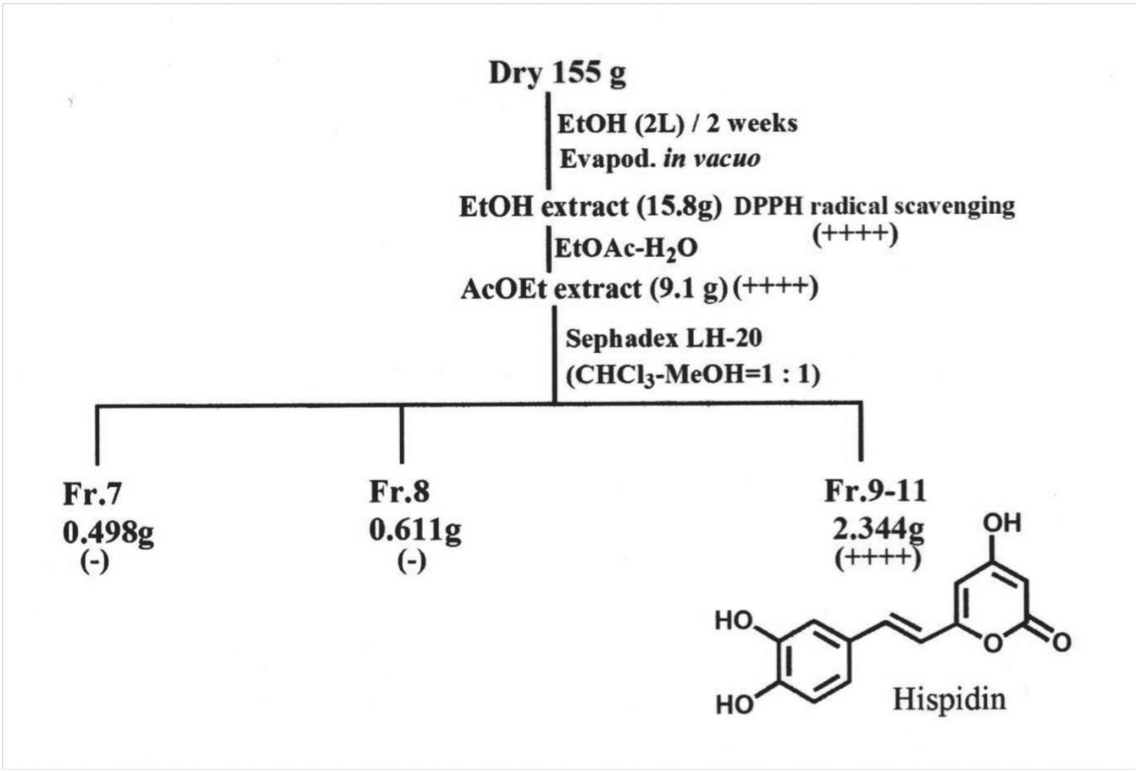

Fig.2. Extraction procedure of Phaeolus schweinitzii

The physicochemical properties of compound 1 are as follows :

HRMS m/z 246.0492 (M^+, $C_{13}H_{10}O_5$, calcd. as 246.0525).

EIMS (200eV) m/z (%): 246 (M^+, 100), 228 (8), 218 (7), 202 (31), 187 (12), 176 (43), 163 (39), 148 (20).

UVλmax (EtOH) nm (logε): 221 (4.49), 252 (4.13), 370 (4.37).

FIIRνmax (KBr) cm^{-1} : 3090 (OH), 1660 (C=O), 1600, 1125, 815.

^{1}H-NMR (acetone-d_6) : δ5.39 (1H, d, J = 2.0Hz, H-3) , 6.14 (1H, d, J=2.0Hz, H-5), 6.68 (1H, d, J= 16.0 Hz,

H-7),6.86 (1H, d, J= 8.1Hz, H-5'), 7.03 (1H, dd, J= 2.0, 8.1Hz, H-6'), 7.15 (1H, d, J=2.0Hz, H-2'), 7.28 (1H, d, J=16.0Hz, H-8).

^{13}C-NMR (acetone-d_6) : δ90.6 (d, C-3), 101.1 (d, C-5), 114.7 (d, C-2'), 116.3 (d, C-7), 117.3 (d, C-5'), 121.6 (d, C-6'), 128.6 (s, C-1'), 136.1 (d, C-8), 146.3 (s, C-4'), 147.9 (s, C-3'), 161.2 (s, C-6), 164.8 (s, C-2x), 171.3 (s, C-4).

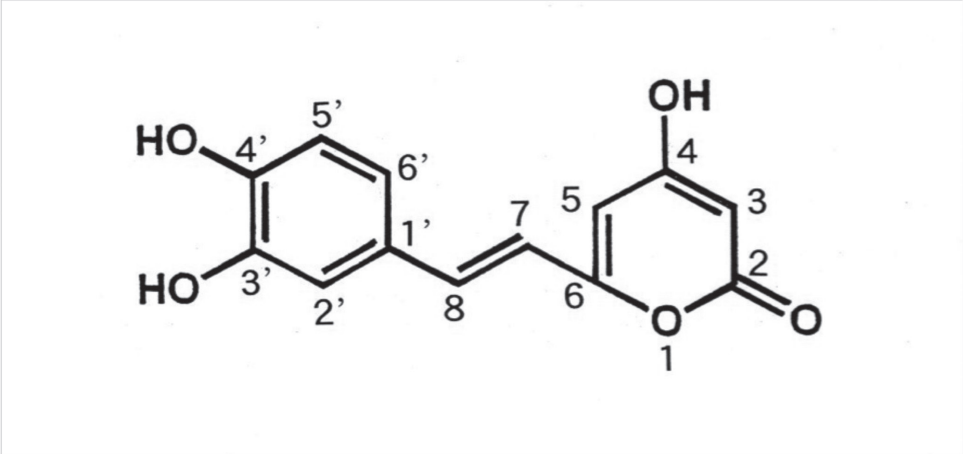

Fig.3. Structure of Hispidin.

Antioxidative activity of compound 1

The antioxidative activity was assayed by measurement of radical-scavenging activity[4].

An aliquot of antioxidant solution (50μM) or ethanol (2ml) was mixed with the 100mM sodium acetate buffer (pH5.5, 2ml) and then added to 1ml of 500μM diphenyl-*p*-picrylhydrazyl (DPPH) in ethanol (final concentration of 100μM) .

The mixture was shaken vigorously and allowed to stand for 30 min. at room temperature in the dark.

The absorbance at 517nm by DPPH was measured by a UV-VIS spectrophotometer (Shimadzu).

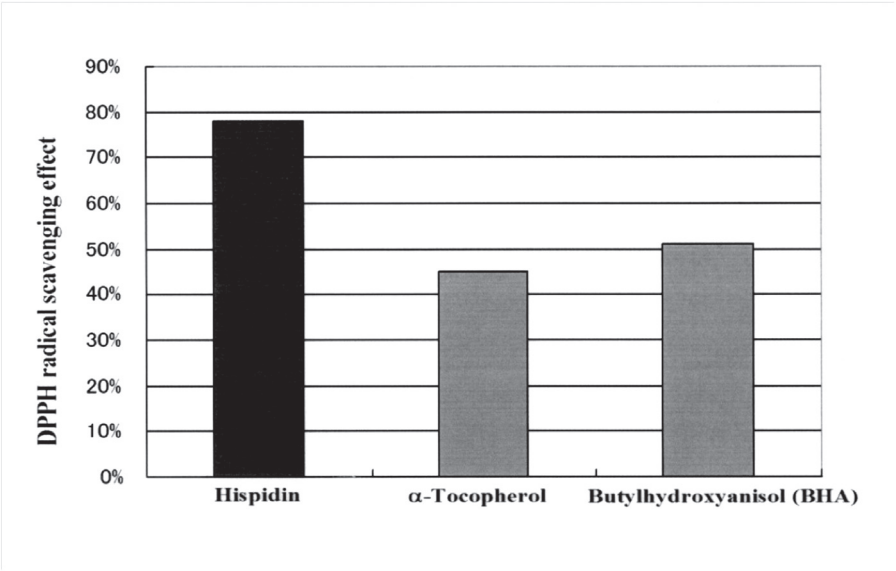

Fig.4. Free radical scavenging effect of hispidin from *Phaeolus schweinitzii* by diphenyl-p-picryl hydrazyl (DPPH) method. α-Tocopherol and BHA were used as the standard antioxidants. The test was run in triplicate and averaged.

Acknowledgments

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Reference

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