Characteristic Long-Chain Fatty Acid of

_Pleurocybella porrigens_

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As part of an investigation on the chemical constituents and contaminants of the basidiomycete _Pleurocybella porrigens_ (Japanese name: Sugihiratake), we analyzed the UV-detected constituents of this mushroom using HPLC. One of the major UV peaks detected was isolated and identified as a-eleostearic acid, a long-chain fatty acid with a conjugated triene moiety, based on the results of spectroscopic methods. a-Eleostearic acid was concluded to be a characteristic fatty acid of _P. porrigens_, because it was not detected in eight other edible mushrooms examined. Free long-chain fatty acids in _P. porrigens_ and other edible mushrooms were analyzed by HPLC after derivatization with acidic 2-nitrophenylhydrazine hydrochloride. Oleic acid was the main fatty acid in _P. porrigens_, and saturated long-chain fatty acids such as linoleic acid, palmitic acid, and stearic acid, together with a-teleostearic acid, were also detected.

**Key words:** Pleurocybella porrigens; Sugihiratake; long-chain fatty acid; a-eleostearic acid

**Introduction**

_Pleurocybella porrigens_ (Japanese name: Sugihiratake) is a basidiomycete of the Tricholomataceae family, and grows naturally on tree stumps and fallen trees such as Japanese cedar (_Cryptomeria japonica_) during the late summer and autumn. This edible mushroom has long been consumed as an autumnal delicacy mainly in the Tohoku and Hokuriku districts of Japan. Since the autumn of 2004, several unknown acute encephalopathy cases have been diagnosed in the Tohoku and Hokuriku districts1). In this study, we investigated the UV-detected constituent of this mushroom and other edible mushrooms.

**Materials and Methods**

**General**

1H- and 13C-NMR spectra were recorded on a JEOL ECA-500 instrument, in chloroform-d (CDCl3) as the solvent. The chemical shifts are given in δ (ppm) based on the solvent signals (δH 7.28; δC 77.0) in tetramethylsilane (TMS). A Waters Micromass ZMD mass spectrometer was used for electrospray ionization (ESI)-MS measurements. High-resolution (HR)-ESI-MS measurements were recorded on JEOL JMS-T100LC mass spectrometer. Analytical HPLC was performed at 40°C as follows: (Condition 1) column: L-column ODS (5 µm, 150 × 4.6 mm i.d., Chemicals Evaluation and Research Institute, Tokyo, Japan), mobile phase: acetonitrile (CH3CN)–3% acetic acid (CH3COOH) in water (85:15) at a flow rate 1.0 mL/min, and detection at 280 nm; (Condition 2) column: YMC-Pack FA (250 × 6.0 mm i.d., YMC Co., Ltd., Kyoto, Japan), mobile phase: CH3CN–water (85:15) [pH 4–5 adjusted by 1 mol/L HCl] at a flow rate 1.2 mL/min, and detection at 400 nm. Preparative HPLC was conducted at 40°C on an L-column ODS (5 µm, 250 × 10 mm i.d.). Column chromatography was performed with MCI-gel CHP 20P (75–150 µm, 300 × 11 mm i.d., Mitsubishi Chemical Corporation, Tokyo, Japan). Standards of long-chain fatty acids [linoleic acid (min. 88%), oleic...
acid (min. 99%), stearic acid (99%), palmitic acid (95%), and α-leostearic acid (>98%) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) or Funakoshi Co., Ltd. (Tokyo, Japan). An assay kit for the analysis of free long-chain fatty acids was purchased from YMC Co., Ltd. All other chemicals were of analytical reagent grade.

The P. porrigens samples were collected during the fall of 2004 or 2005 from local health centers and the prefectural institutes of Public Health and Environment in Japan through the Ministry of Health, Labour and Welfare of Japan. Other mushrooms [Flammulina velutipes (Enokitake in Japanese), Pleurotus eryngii (Eringi), Hypsizygus marmoreus (Bunashimeji), Lyophyllum decastes (Hatakeshimeji), Pleurotus ostreatus (Hiratake), Grifola frondosa (Maitake), Agaricus bisporus (White mushroom), and Lentinula edodes (Shiitake)] were purchased from supermarkets in Tokyo, Japan.

Analysis and isolation of UV-sensitive fatty acids

(1) Analysis. Each fresh mushroom sample was homogenized, and the homogenate (10 g) was extracted with methanol (CH₃OH) (50 mL) by sonication for 20 min. After filtration, the concentrated filtrates were used for HPLC analysis.

(2) Isolation. A fresh sample of P. porrigens (ca. 400 g) was homogenized and extracted with CH₃OH (2 L) by sonication for 20 min, and then filtered. The filtrate was concentrated in vacuo. The concentrated solution was applied to a MCI-gel CHP 20P column with CH₃OH-water (2 : 8) (10), 130.6 (C-13), 131.9 (C-9), 132.9 (C-12), 135.3 (C-14), 14.0 (C-18), 15.0 Hz, H-14), 5.99 (1H, br t, J=10.9 Hz, H-10), 6.09 (1H, dd, J=10.9, 15.0 Hz, H-13), 6.16 (1H, dd, J=10.9, 15 Hz, H-12), 6.39 (1H, br dd, J=10.9, 15.0 Hz, H-11). 13C NMR [in CDCl₃, 126 MHz]: δ 14.0 (C-18), 22.3 (C-17), 24.1 (C-3), 27.9 (C-8), 29.08, 29.11, 29.20 (C-4-6), 29.7 (C-7), 31.5 (C-16), 32.6 (C-15), 34.1 (C-2), 126.0 (C-11), 128.8 (C-10), 130.6 (C-13), 131.9 (C-9), 132.9 (C-12), 135.3 (C-14), 180.0 (C-1).

Fig. 1. HPLC profiles of P. porrigens (Sugihiratake) and other mushroom extracts and UV spectrum of the arrowed peak. A, F. velutipes (Enokitake); B, P. eryngii (Eringi); C, H. marmoreus (Bunashimeji); D, L. decastes (Hatakeshimeji); E, P. ostreatus (Hiratake); F, G. frondosa (Maitake); G, A. bisporus (White mushroom); H, L. edodes (Shiitake). HPLC was performed under "Condition 1" described in "Materials and Methods".

Analysis of free long-chain fatty acids in mushrooms

Free long-chain fatty acids in mushrooms were converted into their 2-nitrophenylhydrazides by using an assay kit based on the methods of Miwa et al. and Miwa and Yamamoto, and the concentrations were estimated by HPLC with detection at 400 nm.

Results and Discussion

We first conducted a constituent analysis of the CH₃OH extract of P. porrigens in order to identify any UV-absorbing compounds present. Figure 1 shows the HPLC chromatograms detected at 280 nm of the extracts from P. porrigens and eight other edible mushrooms [F. velutipes (Enokitake), P. eryngii (Eringi), H. marmoreus (Bunashimeji), L. decastes (Hatakeshimeji), P.
ostreatus (Hiratake), G. frondosa (Maitake), A. bisporus (White mushroom), and L. edodes (Shiitake). A peak characteristic of conjugated trienes, as shown in Fig. 1, was detected in the P. porrigens extract, but not in the extracts of the other edible mushrooms. This peak was also observed in the HPLC eluates of an aqueous extract of P. porrigens obtained by refluxing in water for 30 min. We isolated this compound as a white solid after column chromatography. The HR-ESI-MS data showed an ion peak corresponding to the molecular formula C18H30O2. The NMR spectrum showed the characteristic spectral pattern of a straight-chain fatty acid with a conjugated triene moiety. Spectral analysis by 2D NMR (1H–1H COSY, HQMC, and HMBC) and the characteristic UV absorption pattern lead to the conclusion that this compound was α-eleostearic acid (9,11,13-cis, trans, trans-octadecatrienoic acid)[10,11]. The chemical structure is shown in Fig. 2.

α-Eleostearic acid (EA) was first found as the main fatty acid (about 80% of total fatty acids) of tung oil[12,13]. Conjugated fatty acids such as EA are known to be present mainly in plant seed oils[14]. Several other edible mushrooms were examined and confirmed to not contain this conjugated fatty acid, as described above (Fig. 1). Therefore EA is regarded as a characteristic fatty acid of P. porrigens. Tung oil, which is derived from seeds of the tung tree (Aleurites fordii, Euphorbiaceae), is commonly used in formulations of inks, dyes, coatings, and resins because of its unique ability to dry to a clear, hard finish[11]. Tung nut containing EA is also known to be poisonous, causing a vomiting and diarrhea[16]. However, there have been few reports on the toxic effects of EA. Indeed, EA was recently reported to have potential anticancer applications[16–18].

Free long-chain fatty acids in P. porrigens and other edible mushrooms were also analyzed by HPLC after derivatization to UV-absorbing compounds by treatment with acidic 2-nitrophenylhydrazine hydrochloride. A comparison of the chromatograms among the mushrooms is shown in Fig. 3. Unlike the other mushrooms, the P. porrigens extract contained mainly oleic acid, followed by palmitic, linoleic, stearic acids, and EA. The HPLC patterns of various samples of P. porrigens collected from different areas showed similar compositions. However, these acids, especially oleic acid, are encountered in many other natural foods, and if they are the cause of the acute encephalopathy, it is not likely that this outbreak was caused only by ingestion of P. porrigens.

In conclusion, P. porrigens was revealed to contain a unique unsaturated fatty acid, EA, which was not detected in other edible mushrooms examined in this study. In HPLC analysis of free long-chain fatty acids in P. porrigens, oleic, linoleic, palmitic, and stearic acids were detected, along with EA.

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References


18) Tsuzuki, T., Tokuyama, Y., Igarashi, M., Miyazawa, T., Tumor growth suppression by α-eleostearic acid, a linolenic acid isomer with conjugated triene system, via lipid peroxidation. Carcinogenesis, 25, 1,419–1,425 (2004).