Mercaptan-Capturing Properties of Mushrooms

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Mercaptan-capturing properties of 33 kinds of mushrooms were measured. The mushrooms having a high capturing ability toward methyl mercaptan (MeSH) were *Agaricus bisporus, A. campestris, Boletus fraternus, B. subvelutipes, Gyrodon lividus, Leccinum scabrum, Suillus grevillei, Morchella esculenta, Russula nigricans, Hypholoma sublateritium,* and *Lyophyllum sykosporum*. These are liable to change their color when injured. The mixture of their acetone powders, which contain polyphenol oxidases, and phenolic compounds such as tyrosine, γ-L-glutaminyl-4-hydroxybenzene (GHB), DOPA, variegatic acid, grevillin B and C, and pigments, and fluorescent compounds from *H. sublateritium* also showed high MeSH-capturing properties. 2,5-Bis(methylthio)-DOPA was isolated from the reaction mixture of tyrosine and MeSH with tyrosinase, and the existence of 2- and 5-methylthio-DOPAs was also suggested. Furthermore, acetone powders from fruits and vegetables oxidized the above diphenolic compounds to bind MeSH.

Keywords: *Enzymatic deodorization; mercaptan capture; mushroom; pigment; acetone powder; bad breath; polyphenolic compound; polyphenol oxidase; methyl mercaptan*

INTRODUCTION

Recently we have proposed an enzymatic deodorization method (*1, 3, 4*) that is more effective than the method already known (*2*) to remove bad odors from the environment including bad breath. The principle of enzymatic deodorization is indicated in Figure 1. The *o*-quinones produced from polyphenolic compounds (PP) by polyphenol oxidase (PPO) bind methyl mercaptan (MeSH), which is a bad odor. The bad odors are then removed from the reaction system, and the deodorization is achieved instead of the browning by polymerization of *o*-quinones. In the enzymatic deodorization, it is important that the enzyme accelerates the oxidation reaction, which is the rate-limiting step in deodorization. In previous papers, we demonstrated that bad breath was removed only by eating raw fruits, vegetables, and mushroom (*Agaricus bisporus*) (*3*) and that variegatic acid, which is a pigment isolated from *Boletus subvelutipes*, has a very high potential for deodorization (*4*). Further investigation on the enzymatic deodorization with many kinds of mushrooms and their pigments has been done. In this paper, we report their MeSH-capturing properties.

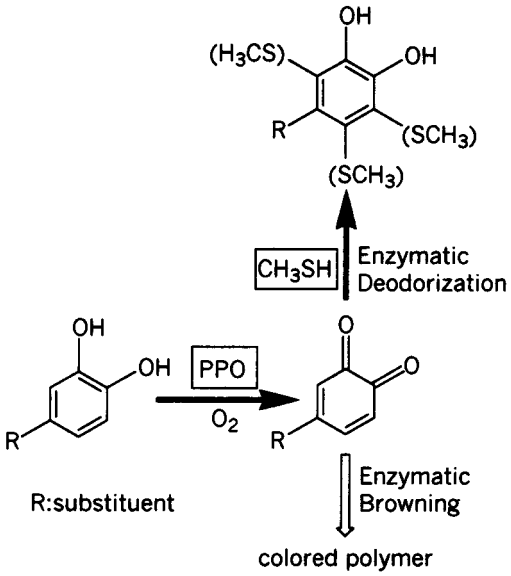

Figure 1. Enzymatic deodorization and enzymatic browning. In the reaction system leading to browning, the *o*-quinones capture MeSHs. Polyphenol oxidase (PPO) accelerates the oxidation reaction.

MATERIALS AND METHODS

Mushrooms. Mushrooms were collected from the fields and mountains in Yamanashi prefecture, Fukushima prefecture, Nagano prefecture, and Ibaraki prefecture, Japan. Some were obtained from the markets. The mushrooms were lyophilized, milled, and stored at −20 °C.

Chemicals. A 15% MeSNa aqueous solution and detector tubes were purchased from Tokyo Chemical Industry Co., Ltd., and Gastec Corp., Japan, respectively. In the detector tubes (5 × 130 mm), silica gel-adsorbed $PdSO_4$ is packed, and when mercaptan gas passes through the tube, the following reaction occurs: $2CH_3SH + PdSO_4$ (white color) → $(CH_3S)_2Pd$ (yellow color) + H_2SO_4. The amount of mercaptan gas is indicated as the length of yellow bar. Tyrosinase (EC 1.14.18.1, 2140 units/mg) from *Agaricus bisporus* is a product of Sigma-Aldrich Corp.

Extractions and Isolations of Phenolic Compounds and Preparations of Acetone Powders from Mushrooms.

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Several phenolic compounds were extracted and isolated from *A. bisporus*, *S. grevillei*, and *H. sublateritium*, and their acetone powders were prepared as follows.

(*i*) *γ-L-Glutaminyl-4-hydroxybenzene* (*GHB*). Raw fruit bodies (1 kg) of *A. bisporus* were homogenized with acetone (3.2 L, 4 °C) in a Waring blender. The homogenate was passed through a filter paper under suction, and the residue was re-homogenized 2 times with 80% acetone (2 L, 4 °C). The solution was filtered, and the filtrates were combined and concentrated to ca. 80 mL. The pH of the solution was adjusted to 3.5 with concentrated HCl, and the precipitate was removed by filtration. Half of the filtrate was subjected to a Sephadex G-25 column (3 × 83 cm). The elution was carried out with water, and the absorbances of the eluates at both 245 and 275 nm were monitored. Additionally, a part of the eluates was incubated with tyrosinase, and absorbances of the reaction mixtures were measured at 460 nm. The red fractions, which mainly contain Tyr (λ_{max} = 275 nm) were eluted, followed by elution of GHB (λ_{max} = 245 nm). The fractions containing GHB were pooled and lyophilized. Crude GHB was further purified with a DEAE-Sephadex A-25 column (1.6 × 35 cm) equilibrated with 0.01 M NH_4HCO_3. GHB was eluted with 0.02 M NH_4HCO_3 after Tyr was eluted with 0.01 M NH_4HCO_3. The fraction containing GHB was lyophilized, and GHB was recrystallized from water. The crystal (45 mg) of GHB was obtained in the form of prisms from 1 kg of raw *A. bisporus*. One mole of this compound yielded 0.98 mol of glutamic acid and 1.0 mol of 4-aminophenol after its hydrolysis with 6 N HCl at 110 °C for 21 h. ^{1}H NMR spectrum of the crystal GHB closely coincided with that reported by Weaver et al. (*5*).

(*ii*) *Grevillin B and C*. The powder (50 g) of lyophilized fruit bodies of *S. grevillei* was extracted with 80% acetone (2 L, 4 °C) by stirring for 1 h. After the solution was filtered, the residue was re-extracted 2 times with the same solvent (1 L, 4 °C) for 30 min. The combined filtrate was concentrated, and the resulting solution was further extracted with *n*-pentane. The water layer was concentrated to remove *n*-pentane and extracted with ethyl acetate. The ethyl acetate layer was evaporated to dryness. Half of the dry residue (0.38 g) was dissolved in 20 mL of MeOH−1% AcOH (40:60) and charged to a Wakosil 25C18 (Wako Pure Chemical Industry, Japan) column (2.5 × 35 cm) equilibrated with the same solvent. Eluates were monitored by measuring the absorbance at 350 nm. Grevillin B and C were clearly separated from other. All the tubes in each fraction were combined and evaporated to dryness and pigments; grevillin B and C were recrystallized from water. The crystals of grevillin B (orange color, 62.5 mg) and C (red orange color, 27.5 mg) were obtained as clusters of needles from 50 g of lyophilized *S. grevillei*. ^{1}H NMR spectra of grevillins closely coincided with that reported by Steglich et al. (*6*).

(*iii*) *Mixture of Phenolic Compounds from H. sublateritium*. An extract from lyophilized *H. sublateritium* with 80% acetone was evaporated to dryness. The dry matter was suspended in MeOH, and the insoluble materials were removed by filtration. The filtrate was evaporated to dryness again. The dry residue was suspended in 100 mL of ethyl acetate containing 2% water. The insoluble matter was removed by filtration, and the filtrate was washed 5 times with 40 mL of 0.1 N HCl. The ethyl acetate layer was washed with water and evaporated to dryness. The dry residue was further dissolved in a small amount of acetone and allowed to stand for some time. The precipitate was removed by filtration, and the filtrate was evaporated to dryness. The dry matter (0.8 g) was obtained from 50 g of lyophilized fruit bodies of *H. sublateritium*.

(*iv*) *Acetone Powders*. The residue after the extraction of phenolic compounds with 80% acetone was lyophilized and used as acetone powder of each mushroom. The acetone powders of fruits and vegetable were prepared in the same way. The acetone powders, which contain enzymes, were stored at −20 °C.

Preparation of γ-L-Glutaminyl-3,4-dihydroxybenzene (GDB). The reaction mixture contained 2 mL of 7.5 mM GHB in 0.02 N HCl, 4 mL of 0.1 M acetate buffer (pH 5.0), and 1 mL of enzyme solution containing 5 mg of tyrosinase. This reaction was carried out at 30 °C for 25 min, and 0.5 mL of 50 mM sodium ascorbate solution was added to the reaction mixture to reduce *o*-quinone structure. These procedures were repeated three times for the treatment of 10 mg of GHB. The AcOH concentration in the combined reaction mixture was adjusted to 1% by addition of AcOH, and the mixture was charged to a Toyopearl HW-40S (Tosoh Corp., Japan) column (1.6 × 34 cm) equilibrated with 1% AcOH. Elution was carried out with 1% AcOH, and the absorbances of the eluates at 245 nm were monitored. The main fraction was pooled and lyophilized. This compound was identified as GDB by comparing its UV spectrum to the preparation in the literature (*7*). Yield of the light-orange colored powder was 12.8 mg, and the purity was 45.2% as calculated from the absorption coefficient.

Measurement of the Mercaptan-Capturing Property. To a mixture of lyophilized mushroom powder (100 mg) and 5 mL of 0.1 M phosphate buffer (pH 7.0) or acetate buffer (pH 5.0) in a 30-mL borosilicate glass vial with open-top screw cap and Teflon/silicon disk (Pierce), 20 μL of a 1.5% MeSNa aqueous solution was added, and the vial was shaken by hand at the rate of 2 strokes per second at 25 °C. After 10 min, 10 mL of the headspace gas was passed through a detector tube (*3*) to measure the amount of remaining methyl mercaptan gas.

The effect of acetone powder and PP was measured by mixing 20 mg of acetone powder, 1.7 mL of 0.1 M phosphate buffer (pH 7.0), 0.2 mL of 5 mM PP solution, and 0.1 mL of a 0.1% MeSNa aqueous solution. After 5-min reaction at 25 °C, 3 mL of the headspace gas was passed through the detector tube.

Control reaction was carried out without a lyophilized mushroom powder or an acetone powder. The mercaptan-capturing property of each material was measured in duplicate. MeSH capture (%) was expressed as $(C - P)/C \times 100$, where C is the amount of MeSH in the control reaction and P is the amount of MeSH in the reaction with the powder.

Large-Scale Reactions and Separation of Conjugates. The reactions were done on a large scale to identify the reaction products between Tyr or GHB and MeSH. In the case of reaction between Tyr and MeSH, the reaction mixture contained 3 mL of Tyr solution (5 mg in 3 mL of 0.02 N HCl), 16 mL of 0.5 M HEPES−KOH (pH 7.0), 150 μL of 15% MeSNa, and 1.5 mL of tyrosinase solution (10 mg in 1.5 mL of the above buffer). The mixture was stirred for 10 h at 30 °C. An additional 3 mL of Tyr solution was added to the mixture after 3 h of reaction. Furthermore, 40 μL of 15% MeSNa solution was also added every hour from the third hour up to the sixth hour of reaction. The reaction was stopped by adding 0.6 mL of concentrated HCl. The reaction product was purified by using a Bond Elut C18 cartridge (500 mg, Varian). After adsorption and washing with water, a stepwise elution with MeOH from 5% to 100% was carried out. The eluates were analyzed by HPLC, and the main product was eluted by 10% MeOH. The fraction containing the conjugate was concentrated and lyophilized. This powder (4.1 mg) was analyzed by NMR.

The reaction between GHB and MeSH was carried out as described for the reaction between Tyr and MeSH. However, no reaction product was isolated because a little amount of specific conjugates accumulated in the reaction between GHB and MeSH with tyrosinase.

Quantitative Analysis of Hydroxybenzene Derivatives in Mushrooms. Hydroxybenzene derivatives were extracted 3 times from 0.5 g of lyophilized mushroom powder with 20 mL of EtOH−0.1 N HCl (70:30). This mixture was well dispersed by the ultrasonic wave treatment for 10 min, followed by standing for 50 min. The mixture was filtered, and the filtrate was concentrated to less than 20 mL. After further filtration, the extract was made up to 25 mL with 0.1 N HCl. A total of 5 μL of this solution was analyzed by HPLC equipped with TSKgel ODS-80Ts column. The analyses were carried out at both 245 and 275 nm wavelengths.

HPLC Analysis. Phenolic compounds and the conjugates with MeSH were analyzed by HPLC (LC-10A system, Shimadzu Corp., Japan) with an Inertsil prep-ODS column (6.0 × 250 mm, 10 μm, GL Sciences, Japan) or an TSKgel ODS-

Table 1. Mercaptan-Capturing Properties of Mushrooms

mushroom	MeSH capture[a] (%) pH 5.0	pH 7.0	mushroom	MeSH capture[a] (%) pH 5.0	pH 7.0
Agaricaceae			Pleurotaceae		
Agaricus bisporus[b]	50	100	*Lentinus edodes*[b]	0	7
A. brazei[b]	10	9	*Pleurotus ostreatus*[b]	7	6
A. campestris	0	100	*P. pulmonarius*[b]	11	0
A. subrutilescens	42	58	*P. salmoneostramineus*[b]	0	4
Phaeolepiota aurea	0	4	Polyporaceae		
Amanitaceae			*Grifola frondosa*[b]	0	2
Amanita hemibapha	3	11	Russulaceae		
Bolbitiaceae			*Lactarius hatsudake*	7	15
Agrocybe cylindracea[b]	4	0	*Russula nigricans*	100	100
Boletaceae			Strophariaceae		
Boletus calopus	50	22	*Hypholoma sublateritium*	100	100
B. fraternus	47	100	*Pholiota nameko*[b]	0	9
B. subvelutipes	100	100	Thelephoraceae		
Gyrodon lividus	100	100	*Sarcodon aspratus*	7	4
Leccinum scabrum	100	67	Tricholomataceae		
Suillus bovinus	0	0	*Flammulina velutipes*[b]	4	0
S. grevillei	100	15	*Hypsizigus marmoreus*[b]	3	6
S. spectabilis	9	0	*Lyophyllum sykosporum*	27	90
Coprinaceae			*Pleurocybella porrigens*	26	2
Coprinus comatus[b]	27	0	*Tricholoma giganteum*[b]	4	4
Morchellaceae			Tuberaceae		
Morchella esculenta	0	100	*Tuber melanosporum*[b]	13	9

[a] Reaction was carried out at 25 °C for 10 min using 100 mg of powder of lyophilized mushroom, 5 mL of 0.1 M acetate or phosphate buffer, and 20 μL of 1.5% sodium methyl mercaptan solution. [b] These are cultivated mushrooms.

80Ts column (4.6 × 250 mm, 5 μm, Tosoh Corp., Japan) at 30 °C. Separation of phenolic compounds from *H. sublateritium* was performed with an Inertsil prep-ODS column and MeOH−1% AcOH (40:60 for 40 min, followed by a linear gradient to 100% MeOH for 20 min and finally holding at 100% MeOH for 20 min). In the case of MeSH conjugates, the same column was used, but the ratio of MeOH to 1% AcOH was 20:80. In both cases, the flow rate was 1.0 mL/min. Retention times of Tyr, GHB, DOPA, and GDB were 11.5, 15.9, 8.7, and 11.0 min, respectively, with an TSKgel ODS-80Ts column and H_2O−MeOH−AcOH−Et_3N (97:3:0.3:0.3) at a flow rate of 0.6 mL/min. The spectra of the eluates were measured using a photodiode array UV−vis detector, SPD-6MA (Shimadzu Corp., Japan).

NMR Spectrum. The structures of the isolated compounds were elucidated by measuring their NMR spectra in CD_3OD or D_2O containing DCl with a JNM-A400 spectrometer (JEOL, Japan). The methyl resonances of TMS at δ 0 ppm in CD_3OD and *tert*-butyl alchohol at δ1.39 ppm (*5*) in D_2O were used as internal standards.

RESULTS AND DISCUSSION

Mercaptan-Capturing Properties of Mushrooms. MeSH-capturing properties of 33 lyophilized mushrooms were measured at pH 5.0 and pH 7.0. As shown in Table 1, the mushrooms that showed a high capturing property toward MeSH were *Agaricus bisporus, A. campestris, Boletus fraternus, B. subvelutipes, Gyrodon lividus, Leccinum scabrum, Suillus grevillei, Morchella esculenta, Russula nigricans, Hypholoma sublateritium*, and *Lyophyllum sykosporum*. The pH of the reaction mixture influenced the MeSH-capturing properties. In *A. bisporus, A. campestris, B. fraternus, M. esculenta*, and *L. sykosporum*, the properties at pH 7.0 were higher than those at pH 5.0, whereas in *B. calopus, L. scabrum*, and *S. grevillei* the properties at pH 7.0 were smaller than those at pH 5.0. It seems that the differences in the properties at the two pH values are due to differences in the optima pH values for enzymes of each mushroom toward endogenous substrates. Most of mushrooms having high MeSH-capturing properties changed their colors. In the previous paper (*4*), we reported the blueing phenomenon and deodorization activities of Boletaceae mushrooms. *B. calopus, B. fraternus, B. subvelutipes*, and *G. lividus* turned blue immediately when they were touched. On the other hand, *A. bisporus, A. campestris*, and *R. nigricans* first turned red and then black. The colors of *Coprinus comatus* and *L. sykosporum* also changed to black. The color changes are thought to be necessary for the occurrence of MeSH-capturing ability, and phenolic pigments and their oxidases seem to be responsible for this phenomenon (Figure 1).

Phenolic Compounds in Mushrooms. A large number of studies on pigments of fungi have been done (*8*). The isolations of the pigments involved in deodorization reaction were examined. L-4-Hydroxyphenylalanine (tyrosine, Tyr) and γ-L-glutaminyl-4-hydroxybenzene (GHB), which cause reddening in *Agaricus* species (*5*), were isolated from *A. bisporus* by the column chromatographies with Sephadex G-25 and DEAE-Sephadex A-25, and recrystallization. GHB was immediately converted into *o*-quinone structure of γ-L-glutaminyl-3,4-dihydroxybenzene (agaridoxin, GDB) by tyrosinase from *A. bisporus*. One of the objective compounds, variegatic acid (*9*), has already been isolated from *B. subvelutipes* (*4*). It is known that other compounds, involutin (*10*), caffeic acid and 3,4,5-trihydroxybenzaldehyde (*11*), and grebillins (*6*), are contained in *G. lividus, L. scabrum*, and *S. grevillei*, which showed high MeSH-capturing properties as shown in Table 1, respectively. Two orange pigments, grevillin B and C, were isolated from *S. grevillei* by the ODS column chromatography and recrystallization. On the other hand, *H. sublateritium* contains yellow pigments (hypholomin A and B) and fluorescence substances (fasciculin A and B) (*8, 12*). They were extracted as a mixture and used for determination of MeSH-capturing property because their amounts were very small and it was difficult to isolate them. The HPLC analysis shows that there are many compounds having the absorption maximum around 350 nm. These phenolic compounds involved in MeSH capture are indicated in Figure 2.

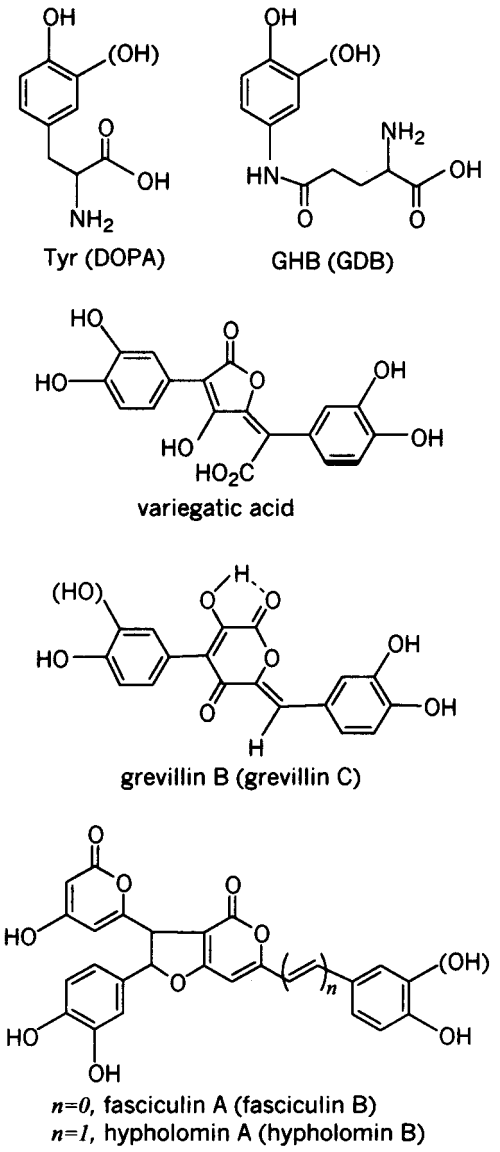

Figure 2. Structures of several phenolic compounds involved in the mercaptan capture.

Table 2. Mercaptan-Capturing Properties of Hydroxybenzene Derivatives with *A. bisporus* Acetone Powder

substrate	MeSH capture[a] (%)
γ-L-glutaminyl-4-hydroxybenzene (GHB)	100
γ-L-glutaminyl-3,4-dihydroxybenzene (GDB)	100
L-4-hydroxyphenylalanine (Tyr)	36
L-3,4-dihydroxyphenylalanine (DOPA)	91

[a] Reaction was carried out at 25 °C for 10 min using 100 mg of acetone powder of *A. bisporus*, 4.5 mL of 0.1 M phosphate buffer (pH 7.0), 20 μL of 1.5% sodium methyl mercaptan solution, and 0.4 mL of 7.5 mM substrate in 0.02 N HCl.

Contents of Hydroxybenzene Derivatives in Mushrooms That Are Liable To Change to Black Color and Their MeSH-Capturing Properties. It is known that *A. bisporus* contains mainly Tyr and GHB as monophenolic compounds and L-3,4-dihydroxyphenylalanine (DOPA) and GDB as minor components (*8*). MeSH-capturing properties of these four compounds with acetone powder were measured. Table 2 shows that the properties of GHB and GDB were very high as compared to Tyr. In the course of oxidation, GHB turned red immediately by treating with the acetone powder and then to black. The contents of these compounds in mushrooms that are liable to change to black color are shown in Table 3. The *Agaricus* species contains a large amount of GHB and particularly *A. bisporus*. In addition, the Tyr content is relatively large in the *Agaricus* species. The mushrooms that contain a large amount of GHB and Tyr showed high MeSH-capturing properties (Tables 1 and 3). The amounts of GHB and Tyr in older *A. campestris* are higher as compared to their contents in the young ones (Table 3). In *C. comatus*, the fruit body became black and melted. In this process, the amount of Tyr increased, but the MeSH-capturing property hardly increased. Ten to twenty times as much Tyr in *Agaricus* mushrooms is contained in *R. nigricans*. Its content was 9.5% of the dry weight in the young fruit body. It has already been reported that some mushrooms contain large amounts of Tyr (*13*), such as *C. comatus* (2500 mg/100 g DW) and *R. rubescens* (1230 mg/100 g DW). The reaction mixture containing a lyophilized powder of *R. nigricans* became deep red in the early period of the MeSH-capturing reaction. *R. nigricans* is one of the mushrooms with very high MeSH-capturing ability. In other mushrooms such as *L. sykosporum* and *M. esculenta*, the amounts of hydroxybenzene derivatives were low, although their MeSH-capturing properties were rather high, suggesting that other compounds were involved in the reactions.

Table 3. Contents of Hydroxybenzene Derivatives in Mushrooms Causing Reddening or Blackening

mushroom	contents (mg/100 g dry weight) GHB	GDB	Tyr	DOPA
Agaricus bisporus	492	50	372	10
A. brazei	16	–[a]	331	–
A. campestris (young)	109	–	371	–
A. campestris (old)	192	106	853	51
A. subrutilescens	112	57	185	62
Coprinus comatus (young)	–	–	350	–
C. comatus (old)	–	–	700	–
Lyophyllum sykosporum	–	–	31	–
Morchella esculenta	–	–	24	–
Pleurocybella porrigens	–	–	72	–
Russula nigricans (young)	–	–	9,530	80
R. nigricans (old)	–	–	6,540	165

[a] –, not detected.

Identification of Conjugates between DOPA and MeSH. The reaction mixture of Tyr and MeSH with tyrosinase was analyzed by HPLC as shown in Figure 3. Some new peaks appeared and could be the three conjugates with MeSH. Their UV spectra (Figure 4) suggested that the conjugates a–c have structures substituted with MeSHs at the 2-, 5-, and 2,5-positions of the benzene ring, respectively, by comparison of the spectra of 2′-(2-hydroxyethylthio)–(−)-epicatechin, 5′-(2-hydroxyethylthio)–(−)-epicatechin, and 2′,5′-bis(2-hydroxyethylthio)–(−)-epicatechin (*14*). The compound c in Figure 3 was separated from the large-scale reaction mixture with ODS column chromatography. NMR spectra data of compound c were compared to those of Tyr and DOPA. In the ^{1}H NMR spectrum, only one signal of H-6 in benzene ring was observed at δ 6.97 ppm (H, s) as compared to two signals derived from H-2, H-3, H-5, and H-6 in the benzene ring of Tyr at δ 7.05 ppm (2H, d) and δ 7.35 ppm (2H, d). Furthermore, there were additions of two -SMe signals at δ 2.40 ppm (3H, s) and δ 2.58 ppm (3H, s), and their binding positions at C-2 and C-5 on the benzene ring were assigned on the basis

Table 4. Substrate Specificities of Several Acetone Powders

acetone powder	MeSH capture[a](%)						
	GHB	Tyr	DOPA	VA	Gre[b]-B	Gre[b]-C	Ext-Hs[c]
apple	0	0	84	100	100	100	26
pear	7	8	100	100	100	100	67
burdock	0	0	19	100	100	100	48
Agaricus bisporus	100	37	88	100	100	100	25
Boletus subvelutipes	19	12	38	100	28	100	25
Hypholoma sublateritium	24	54	100	100	100	100	100
Russula nigricans[d]	84	100	100	100	65	100	100
Suillus grevillei	16	6	13	16	8	17	4

[a] Reaction was carried out at 25 °C for 5 min using 20 mg of acetone powder, 1.7 mL of 0.1 M phosphate buffer (pH 7.0), 0.1 mL of 0.1% sodium methyl mercaptan solution, and 0.2 mL of 5 mM substrate. [b] Grevillin. [c] This is an extract from fruit bodies of *Hypholoma sublateritium*. The concentration of this substrate was 4 mg in 0.2 mL of MeOH. [d] Tyr content was 0.05 mg in 20 mg of acetone powder.

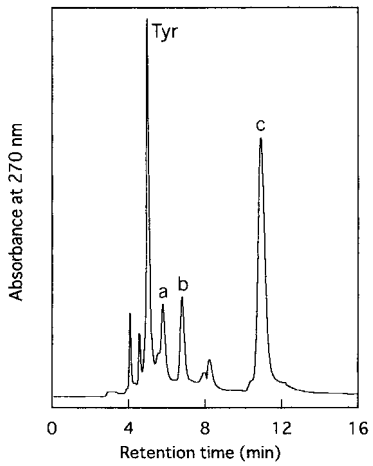

Figure 3. HPLC analysis of reaction products between Tyr and MeSH with tyrosinase. Peaks a−c are the conjugates.

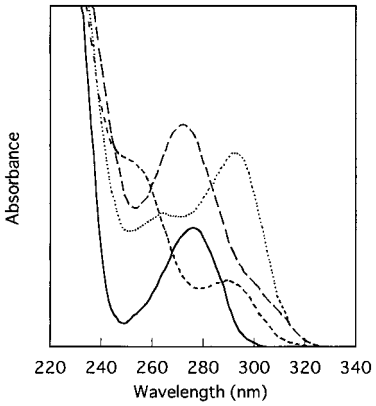

Figure 4. UV spectra of conjugates in Figure 3: —, Tyr; ···, a; ---, b; — —, c. Comparing these spectra with those of conjugates between (−)-epicatechin and 2-mercaptoethanol (*14*), the conjugates a−c are considered to be 2-methylthio-DOPA, 5-methylthio-DOPA, and 2,5-bis(methylthio)-DOPA, respectively.

of HMBC (heteronuclear multiple bond connectivity) spectrum. These data elucidated that the structure of the conjugate (compound c) is 2,5-bis(methylthio)-DOPA.

These results demonstrate that, after the oxidation of substrates by the enzyme, the addition reaction between oxidized substrates and MeSH occurred, and consequently, elimination of MeSH was achieved. In the case where other polyphenolic compounds such as GHB, grevillins, hypholomines, and fasciculines were used, it was speculated that the MeSH capture by the polyphenolic compounds was achieved by the same mechanism as Tyr, variegatic acid (*4*), chlorogenic acid (*1*), and (−)-epicatechin (*14*).

MeSH-Capturing Properties of the Other Compounds and Substrate Specificities for Several Kinds of Acetone Powders. For application of enzymatic deodorization with mushroom pigments for removal of environmental bad odors including bad breath (amines as well as mercaptans), we examined the substrate specificities for several acetone powders toward phenolic compounds (Table 4). The acetone powders from apple, pear, and burdock were used considering their application for removal of bad odors from our mouths. The oxidizing activity of acetone powder from *S. grevillei* toward various substrates including the native compounds was weak, whereas those of the most other mushrooms were very high. Furthermore, toward oxidation of monophenols, acetone powders from *A. bisporus* and *R. nigricans* showed strong activities. Grevillin B and C, whose structures are similar to that of VA, were highly oxidized to capture MeSH by acetone powders of fruit and vegetables other than mushrooms. Although the fruit bodies of *B. subvelutipes*, *S. grevillei*, and *H. sublateritium* are not eaten raw, removal of bad odors by the combination of their pigments and fruits or vegetables can be done as an application of enzymatic deodorization. Consequently, the ability of popular mushrooms to remove bad odors from the environment was proved by this study. Moreover, it was demonstrated that substrate specificities for polyphenol oxidases in mushrooms, especially *R. nigricans*, are relatively low as compared to those in fruits and vegetables or that those mushrooms contain enzymes involved in oxidations of both monophenol and diphenol. Because acetone powders contain almost all the enzymes whose activities are more stable than those of the purified enzyme, many kinds of acetone powders seem to be more useful than purified enzymes as catalysts for the deodorization reaction (Figure 1).

ABBREVIATIONS USED

DOPA, L-3,4-dihydroxyphenylalanine; GDB, γ-L-glutaminyl-3,4-dihydroxybenzene; GHB, γ-L-glutaminyl-4-hydroxybenzene; PP, polyphenolic compound; PPO, polyphenol oxidase; Tyr, L-4-hydroxyphenylalanine; VA, variegatic acid.

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