Identification of Sulfurous Compounds of Shiitake Mushroom (*Lentinus edodes* Sing.)

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Volatile sulfurous compounds of Shiitake mushroom (*Lentinus edodes* Sing.) were extracted from the homogenate of fresh mushrooms, fractionated by silica gel column chromatography, and analyzed by capillary gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). There were 18 noncyclic and cyclic S compounds identified; 13 out of 18 were being reported for the first time as components of Shiitake mushroom. Cyclic S compounds such as lenthionine ($C_2H_4S_5$, 1,2,3,5,6-pentathiepane), 1,2,4,5-tetrathiane ($C_2H_4S_4$), 1,2,3,5-tetrathiane ($C_2H_4S_4$), and 1,2,4-trithiolane ($C_2H_4S_3$) were the major S compounds identified in the mushroom homogenate.

INTRODUCTION

Shiitake (*Lentinus edodes* Sing.) is an edible mushroom highly prized in China and Japan. The fresh mushroom exhibits only a slight odor, but upon drying and/or crushing, a characteristic sulfurous aroma gradually develops (Yasumoto et al., 1976). Lenthionine (1,2,3,5,6-pentathiepane, $C_2H_4S_5$), a cyclic S compound known to possess the characteristic aroma of Shiitake mushroom, was first identified in dry mushroom (Morita and Kobayashi, 1966; Wada et al., 1967). 1,2,4-Trithiolane ($C_2H_4S_3$), 1,2,4,6-tetrathiepane ($C_3H_6S_4$), and 1,2,3,4,5,6-hexathiepane (CH_2S_6) were three other cyclic S compounds also reported in dry product (Morita and Kobayashi, 1967). A recent study of volatiles in dry Shiitake mushroom identified the presence of dimethyl disulfide, dimethyl trisulfide, 1,2,4-trithiolane, and an S compound with molecular formula $C_2H_4S_4$. The presence of lenthionine in the extract of dry mushrooms was confirmed but was considered as decomposed during analysis (Charpentier et al., 1986). Chemical methods to synthesize these cyclic S compounds were also documented (Morita and Kobayashi, 1967). The above-mentioned cyclic S compounds, with the exception of 1,2,3,4,5,6-hexathiepane, were also identified in a species of red algae (*Chondria californica*) (Wratten and Faulkener, 1976). 1,2,4-Trithiolane was identified in the steam distillate of homogenized fresh mushrooms and was the only cyclic S compound reported (Kameoka and Higuchi, 1976; Chen et al., 1984). 1,2,4-Trithiolane has also been reported in the volatile compounds of egg (Gil and MacLeod, 1981) and the reaction product of H_2S with D-glucose (Sakaguchi and Shibamoto, 1978). Iwami et al. (1975a, 1975b, 1975c) and Yasumoto et al. (1976) proposed that cyclic S compounds in Shiitake mushroom were originated from lentinic acid, which is a derivative of γ-glutamylcysteine sulfoxide. There are two enzymes that are responsible for the conversion of lentinic acid into cyclic S compounds: that is, γ-glutamyl transpeptidase and cysteine sulfoxide lyase (C-S lyase). In dry Shiitake mushroom, Ito et al. (1978) noted that the generation of lenthionine was affected by the pH and temperature during rehydration of the dry mushrooms.

The present study reports the identification of S compounds isolated from the homogenate of fresh Shiitake mushrooms.

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EXPERIMENTAL SECTION

Fresh Shiitake mushrooms (*Lentinus edodes* Sing.) were obtained daily from a local cultivation house near Hsinchu, Taiwan. Preparation of samples to be analyzed was the same as described (Chen and Ho, 1986). Fresh mushrooms (100 g) were blended at room temperature with distilled water (500 mL) for 3 min in a Waring blendor; the pH during blending was adjusted by adding 0.1 N NaOH or 0.1 N HCl solution. Celite (50 g; Wako) was added to aid the separation of solid and liquid fractions. Both fractions were extracted thrice by chloroform (1.5 L, in total, E. Merck), combined, and concentrated to minimal volume at reduced pressure. A control experiment was conducted by blending fresh mushrooms directly with chloroform (1.5 L) at room temperature in order to inactivate the enzymic activities. The S fraction was eluted by *n*-hexane/ether (9/1) (300 mL; glass-distilled, GR; E. Merck) on a glass column (50 cm × 1.5 cm i.d.) packed with silica gel (50 g, C-200; Wako). Dipropyl disulfide (2.2 mg; Wako) was added as internal standard. Carbon disulfide was obtained from Merck. Dimethyl disulfide, dimethyl trisulfide, and dimethyl tetrasulfide were obtained from Wako (reagent grade).

Chemical Syntheses. Lenthionine was synthesized by the method of Morita and Kobayashi (1966). Sodium sulfide (E. Merck), sulfur (E. Merck), and formaldehyde (E. Merck) were used as starting materials. 1,2,4-Trithiolane was synthesized according to the method of Gil and MacLeod (1981).

GC. A Shimadzu (Tokyo, Japan) GC-8APTF gas chromatograph was equipped with a flame ionization detector, a capillary injection system (CLH-800, Shimadzu), and a fused silica capillary column (50 m × 0.2 mm i.d., cross-linked OV-1; Hewlett-Packard). The operating conditions were as follows: injector and detector temperatures, 250 °C; hydrogen carrier velocity, 12 cm/s; makeup nitrogen flow, 30 mL/min; detector hydrogen flow, 30 mL/min; detector air flow, 300 mL/min; temperature program, 50–280 °C at 4 °C/min and held at 280 °C for 50 min. Quantitative determinations (without considering response factor of detector, calibration factors $F = 1.00$ for all components) were carried out by using a Shimadzu C-R2A laboratory integrator (Shimadzu). Linear retention indices were calculated by using *n*-paraffins (C_6–C_{22}; Alltech Associates) as references (Majlát et al., 1974).

GC–MS. Identifications of S compounds were conducted on a Hewlett-Packard 5985B GC–MS system. A Hewlett-Packard 5840A gas chromatograph equipped with a fused silica capillary column (50 m × 0.22 mm i.d., CP-SIL 5 CB, equivalent to OV-1; Chrompack) was connected directly into the mass spectrometer. The operating conditions were as follows: injector temperature, 250 °C;

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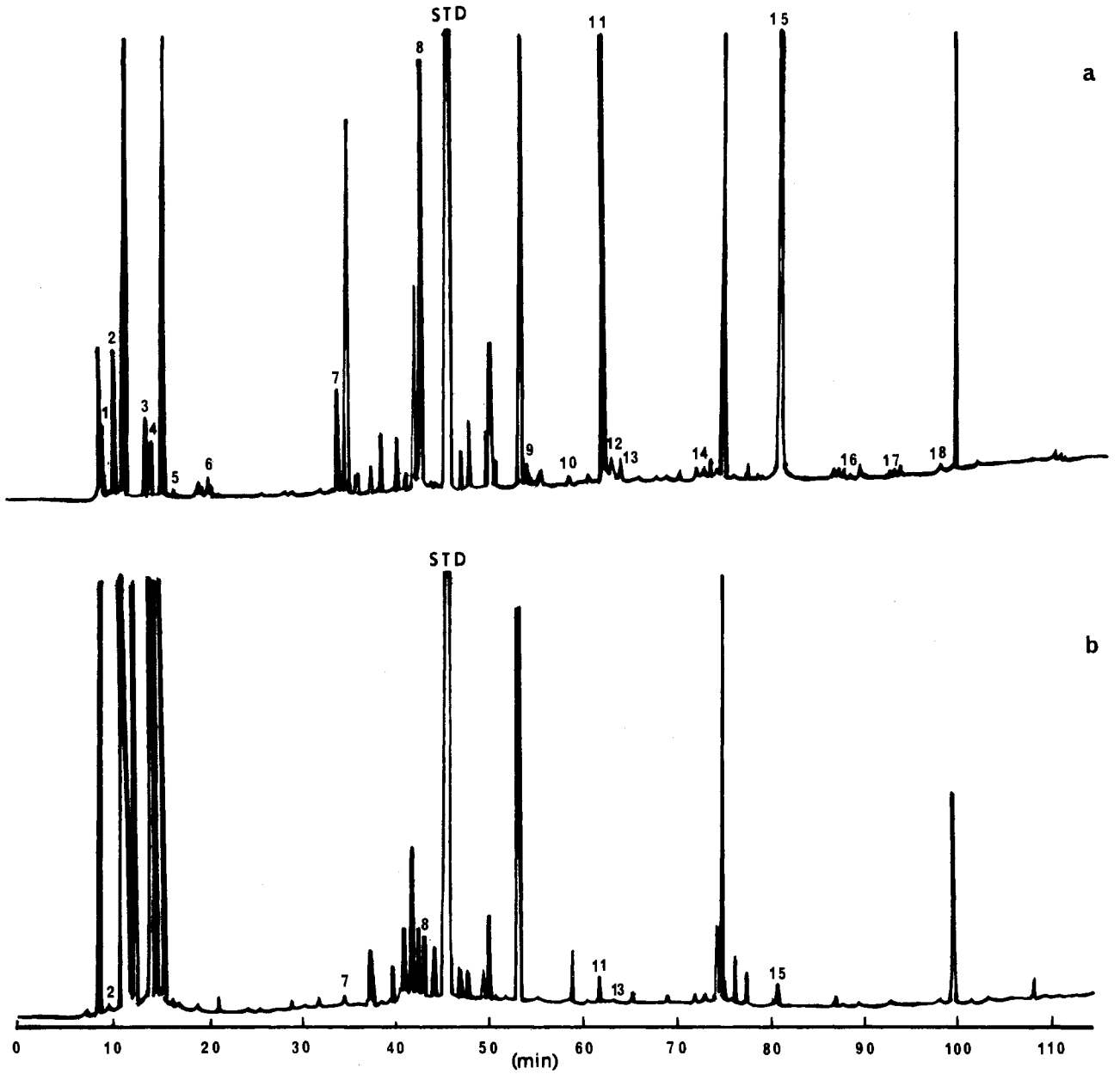

Figure 1. Capillary gas chromatographic separations of S compounds: (a) compounds extracted from pH 9.0 homogenate; (b) control, extracted by chloroform.

temperature program, 50–280 °C at 4 °C/min and held at 280 °C for 50 min; carrier helium velocity, 14 cm/s; ion source and all connection part temperatures, 200 °C; electron energy, 70 eV; electron multiplier voltage, 2600 V.

RESULTS AND DISCUSSION

It is known that sulfurous compounds are susceptible to thermal decomposition (Hiley and Cameron, 1975; Wajon et al., 1985). Wada et al. (1967) also noted that lenthionine will decompose completely if heated at 100 °C in a 10% alcohol solution for 1 h (pH >5.0). In the present study, instead of steam distillation to isolate the sulfurous compounds from Shiitake mushroom (Kameoka and Higuchi, 1976; Chen et al., 1984), solvent extraction of mushroom homogenate followed by column chromatographic fractionation was used.

Figure 1 shows the capillary gas chromatographic separations of S compounds isolated from pH 9.0 homogenate (a) and control (b). Nonpolar stationary phase (OV-1) was chosen for its superior performance in analyzing S-containing compounds (Shu et al., 1985).

The results of GC and GC–MS analyses of S compounds are shown in Table I. Identifications were accomplished by comparing the retention indices and mass spectra with those of authentic compounds and published data (Morita and Kobayashi, 1966, 1967; Kameoka and Higuchi, 1976; EPA/NIH, 1980; Gil and MacLeod, 1981). The structure assignments of some of the novel S compounds were based solely on the interpretation of mass spectral data; therefore, these compounds are considered as tentative identified at present. There were 18 S-containing compounds detected in the pH 9.0 homogenate. The optimal pH for enzymic activities (γ-glutamyl transferase and cysteine sulfoxide lyase) reported was around 9.0 (Iwami et al., 1975a, 1975b, 1975c). The significant quantitative difference detected in the pH 9.0 and the control samples, as shown in Figure 1 and Table I, resulted in the lack of enzymic activities in the latter.

Figure 2 shows the structures of these compounds. The number of S atoms in each S compound could be confirmed by the ratio of $M^+ + 2/M^+$ (4.4% for one S atom),

Table I. Volatile Sulfurous Compounds Identified in Shiitake Mushroom

no.[a]	compound[b]	I,[c] OV-1	MW	%[d] pH 9.0	%[d] control	ID	MS data[e]
1	methanethiol*[f]	613	48	0.62	–	MS	47 (100), 48 (87), 45 (57), 46 (14), 50 (*5*)
2	carbon disulfide*	625	76	4.92	+	GC, MS	76 (100), 78 (*10*), 77 (3), 44 (3)
3	methyl hydrodisulfide*[g]	686	80	0.17	–	MS	80 (100), 76 (16), 64 (16), 82 (*10*)
4	dithiomethane*[g]	692	80	0.16	–	MS	80 (100), 47 (19), 45 (15), 46 (11), 82 (*8*)
5	dimethyl disulfide	745	94	+	–	GC, MS	94 (100), 79 (41), 96 (*10*), 45 (7), 61 (5), 64 (5)
6	1,3-dithietane*[g]	786	92	+	–	MS	92 (100), 94 (*30*), 77 (21), 45 (15), 61 (14), 76 (14), 96 (3)
7	dimethyl trisulfide	949	126	2.72	+	GC, MS	126 (100), 79 (24), 111 (16), 128 (*14*), 80 (10), 64 (7)
8	1,2,4-trithiolane	1065	124	14.04	1.09	GC, MS	124 (100), 78 (71), 45 (32), 126 (*14*), 46 (14), 64 (10), 80 (10)
9	dimethyl tetrasulfide*	1194	158	+	–	GC, MS	158 (100), 79 (73), 64 (22), 94 (22), 160 (*22*), 45 (21), 124 (21)
10	1,3,5-trithiane*[f]	1259	138	+	–	MS	138 (100), 92 (29), 140 (*15*), 46 (14), 45 (12), 91 (12), 64 (10)
11	1,2,4,5-tetrathiane*[h]	1310	156	42.34	0.93	MS	156 (100), 110 (76), 158 (*19*), 112 (10), 64 (8), 45 (7), 78 (6)
12	2,3,5,6-tetrathiaheptane*[g]	1327	172	+	–	MS	93 (100), 126 (26), 79 (17), 172 (13), 45 (11), 110 (8), 64 (5), 174 (*2*)
13	1,2,3,5-tetrathiane*[h]	1338	156	2.18	+	MS	156 (100), 110 (55), 158 (*19*), 91 (13), 78 (12), 45 (10), 124 (10)
14	1,2,4,6-tetrathiepane[i]	1477	170	+	–	MS	170 (100), 124 (88), 78 (83), 45 (19), 172 (*19*), 126 (15), 93 (10)
15	lenthionine	1590	188	39.70	0.63	GC, MS	142 (100), 124 (91), 78 (84), 188 (32), 110 (21), 45 (18), 190 (*9*)
16	1,2,4,7,9,10-hexathiadodecane*[g]	1701	278	+	–	MS	93 (100), 139 (97), 78 (17), 45 (16), 141 (15), 79 (15), 124 (10)
17	1,2,4,5,7-pentathiocane*[g]	1749	202	+	–	MS	138 (100), 137 (99), 110 (82), 78 (35), 91 (28), 124 (27), 45 (22), 202 (19), 170 (8), 204 (*4*)
18	1,2,3,5,6,8-hexathionane*[g]	1901	234	+	–	MS	188 (100), 110 (74), 78 (65), 234 (52), 142 (52), 124 (36), 45 (30), 156 (25), 170 (25), 236 (22)

[a] Number refers to Figure 1. [b] Compounds with asterisks indicate those newly identified in Shiitake mushroom. [c] Calculated value using *n*-paraffins (C_6–C_{22}; Alltech Associates) as references. [d] Percentage relative to internal standard, dipropyl disulfide (2.2 mg/100 g of mushrooms). [e] Number in parentheses indicates relative percentage; those italicized indicate M + 2. [f] EPA/NIH, 1980. [g] Tentatively identified. [h] Wratten and Faulkener, 1976; Charpentier et al., 1986. [i] Morita and Kobayashi, 1967.

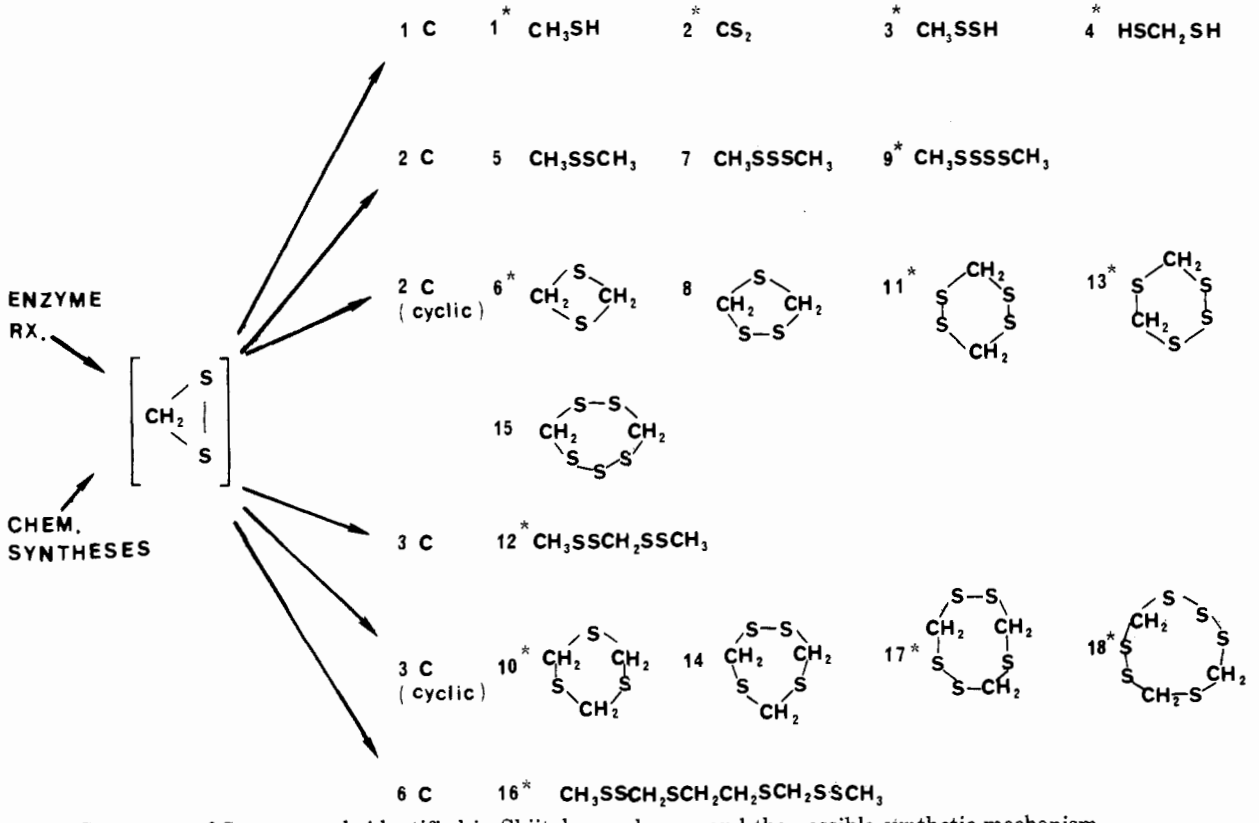

Figure 2. Structures of S compounds identified in Shiitake mushroom and the possible synthetic mechanism.

with the exception of compounds **6** and **16**; all other S compounds listed in Table I exhibit good agreement between the isotope ratio and the number of S atoms. The relatively higher percentage of $M^+ + 2$ of compound **6** may due to the disportionation reaction during the opening of the parent cyclic structure, as can be evidenced by the presence of approximately 3.0% of $M^+ + 4$. The relative percentage of parent ion and $M^+ + 2$ of compound **16** could not be detected in this study; the structure assignment was based on the fragments produced. According to the carbon

number, these S compounds can be classified into four major groups containing 1, 2, 3 and 6 carbons, respectively. Of the 18 S compounds reported in this study, 13 are new to the volatiles of Shiitake mushroom (compounds with an asterisk in Table I).

Carbon disulfide, dimethyl trisulfide, 1,2,4-trithiolane, 1,2,4,5-tetrathiane, 1,2,3,5-tetrathiane, and lenthionine were the dominant S compounds detected when the mushrooms were blended in pH 9.0 buffered solution. It is interesting to note that two isomers—1,2,4,5-tetrathiane and 1,2,3,5-tetrathiane—have been tentatively identified in red algae (*Chondria californica*) (Wratten and Faulkener, 1976). Together with 1,2,4-trithiolane, 1,2,4,6-tetrathiepane, and lenthionine, Shiitake mushroom and red algae have in common five S compounds. It is quite possible to assume that these two different species share the same mechanism that leads to the formation of above-mentioned S compounds. A recent study of volatiles in dry Shiitake mushroom also reported the presence of one of the isomer with molecular formula of $C_2H_4S_4$. Sensory analysis of the eluting peak via sniff port indicated a characteristic Shiitake odor (Charpentier et al., 1986).

With the exception of carbon disulfide (CS_2), all the S compounds listed in Figure 2 have either the $-CH_2S-$ or $-SCH_2S-$ grouping in their structures. The $-SCH_2S-$ functional grouping is similar to methylene disulfide, a proposed building block (or intermediate) for the polymerization of S compounds (Morita and Kobayashi, 1967; Yasumoto et al., 1976).

It is worth noting that all the S compounds identified in the enzymic reaction mixture could also be detected in the products of synthetic reaction of lenthionine or 1,2,4-trithiolane. It is therefore reasonable to assume that chemical reactions (such as the polymerization of the methylene disulfide) may be the dominant forces in the final stages of S-compound formation. Morita and Kobayashi (1967) also noted that pH would affect the composition of S compounds formed during chemical synthesis.

The scheme shown in Figure 2 summarizes the results of the present study and previous reports (Morita and Kobayashi, 1967; Yasumoto et al., 1976; Charpentier et al., 1986) about the formation of S compounds in Shiitake mushroom. Methyl disulfide is proposed as an important intermediate for the polymerization of S compounds listed in Figure 2. Formation of methyl disulfide can be via an enzymic process (Iwami et al., 1975a, 1975b, 1975c; Yasumoto et al., 1976) or chemical process (Morita and Kobayashi, 1966, 1967; Gil and MacLeod, 1981).

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