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ISOLATION AND SYNTHESIS OF LENTHIONINE, AN ODOROUS SUBSTANCE OF SHIITAKE, AN EDIBLE MUSHROOM.*

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A certain species of edible mushroom, Shiitake Lentinus edodes (Berk.) Sing , is highly prized as comestible in Asian countries including Japan and China.

The ingredient of the taste of the mushroom has recently been characterized as guanosine-5'-monophosphate (1), the compound now being produced on a commercial scale in this country.

This report deals with the isolation from the mushroom of the characteristic odorous matter, which we named Lenthionine, and its total synthesis from simple starting materials.

Dried mushroom (5 kg.) which was purchased from market was immersed in water overnight. During this period the characteristic odor of the mushroom evolved; this fact is reminiscent of that the odorous substance is formed from an unknown precursor in the mushroom by the action of certain enzyme. The mixture was centrifuged in a basket centrifuge, and solid material was extracted with methylene chloride (90 kg.)

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The methylene chloride extract was separated and the solvent evaporated under reduced pressure to yield a light yellow oil (21.7 g.). Careful chromatography of this oil on silica gel with chloroform as solvent furnished the following fractions: fraction-1 (1.58 g.), fraction-2 (4.68 g.), fraction-3 (1.72 g.), fraction-4 (1.74 g.), fraction-5 (2.78 g.), and fraction-6 (1.96 g.).

From fraction-6 was obtained an odorless substance, m.p. 141-142°, $C_{18}H_{34}S_4O_4$, of which the structural investigation is under progress.

Fraction-1 was re-chromatographed on silica gel with n-hexane as solvent to yield a pale yellow oily substance which gave a single spot on thin layer chromatography with four different solvent system.

On standing in an ice box this oily substance gradually crystallized. The crystal (0.44 g.) thus obtained, after recrystallization from methylene chloride, melted at 60-61° and had the characteristic odor of the mushroom.

The high resolution mass spectrum of this compound indicated the molecular composition to be $C_2H_4S_5$ (M^+: found 187.8890, Calcd. 187.8917). Determination of the molecular weight by the osmotic pressure method also gave the value of 188 and the elementary analysis was in good accord with the value calculated on the proposed formula. Anal. Calcd. for $C_2H_4S_5$: C, 12.75; H, 2.14; S, 85.11. Found: C, 12.89; H, 2.22; S, 85.13.

Beside the molecular peak at mass 188, the conventional mass spectrum showed its isotope peak at m/e 190; this latter peak had the relative intensity of about one-fourth of the magnitude of the parent peak. The isotope abundance ratio of an isotope peak against the parent peak in the present instance should reflect the number of sulfur atoms in the fragment. In fact, the observed ratios were in good accord with the theoretical values (TABLE I).

Another valuable information obtained from mass spectrum was that the fragmentation pattern indicated the elimination of $-CH_2-$ and -S- fragments from the parent molecule. Thus the pattern is reasonably explained as shown in TABLE I.

TABLE I

Mass Spectral Data of Lenthionine

m/e	Fragments	Relative Intensities %	Isotope Abundance Ratio %	
			Found	Calculated*
188	$(CH_2)_2S_5$	31	22.3	22.0
174	$(CH_2)S_5$	2	22.5	22.0
156	$(CH_2)_2S_4$	50	18.0	17.6
142	$(CH_2)S_4$	71	17.9	17.6
124	$(CH_2)_2S_3$	41	14.1	13.2
110	$(CH_2)S_3$	33	13.8	13.2
96	S_3	6	13.8	13.2
78	$(CH_2)S_2$	100	9.2	8.8
64	S_2	34	10.0	8.8
46	$(CH_2)S$	63	5.4	4.4
45	CH=S	99	-	-

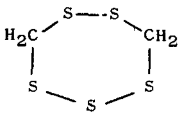
* First order approximation by the equation :

$$\frac{P_M + 2}{P_M} = W \left(\frac{S_{34}}{100 - S_{33} - S_{34} - S_{36}} \right) = W \times \frac{4.22}{95}$$

where W = number of sulfurs in the fragment.

The n.m.r. spectrum of lenthionine provides further evidence that the compound bears a highly symmetric structure with respect to two methylene groups because it exhibits a single peak at $\delta = 4.33$ ppm (singlet), which is ascribed to $-S-CH_2-S-$ grouping.

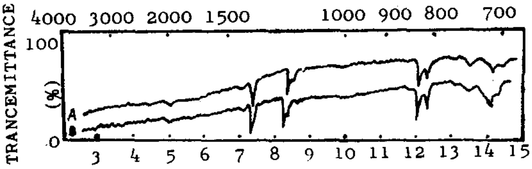
In addition to these arguments, it is worthy of note that the mass due to $-CH_2-S-CH_2-$ fragment was virtually absent in the spectrum of lenthionine, while the peak corresponding to the same fragment was clearly visualized in the spectrum of tri-*thioformaldehyde*. *This* almost certainly excludes the possibility that lenthionine contains a $-CH_2-S-CH_2-$ grouping in the molecule, thus leaving only one possible structure (I) for lenthionine.



(I)

Lenthionine

The IR spectra of natural and synthetic lenthionine



natural lenthionine (A)

synthetic lenthionine (B)

Although the structure proposed appears to be very unusual, support for this was obtained by total syntheses (2) of the compound by the following two methods:

To a solution of $Na_2S_{2.5}$, which was freshly prepared from $Na_2S\cdot 9H_2O$ (30 g.) and sulfur (6 g.) in 100 ml. of water, was added an aqueous 37%-formaldehyde (20 g.) and the solution was covered by 200 ml. of chloroform. The mixture was vigorously stirred at room temperature, while acetic acid (16 ml.) was added in small portions over a period of five hours. The chloroform layer was separated, washed, dried over sodium sulfate and the solvent was evaporated under reduced pressure to yield a crude oily material (450 mg.).

Chromatography of this oil on silica gel as described with natural lenthionine afforded crystals (111 mg.), which was identical with an authentic sample in all respects, including infrared spectrum, thin layer chromatographic behaviors, mixed melting point determination and its characteristic odor.

An alternate synthesis involves the reaction of methylene iodide with $Na_2S_{2.5}$; a mixture of 140 mg. of methylene iodide and $Na_2S_{2.5}$ (126 mg.) in 2 ml. of water was vigorously stirred at room temperature for 5 hours, and the mixture was extracted with chloroform. The chloroform extract was then worked up as described to yield 7 mg. of crystalline lenthionine.

The occurrence of lenthionine from a natural source is undoubtedly the first instance of highly sulfur containing organic compound in nature.

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