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122. Katsura Morita and Shigeru Kobayashi: Isolation,
Structure, and Synthesis of Lenthionine
and Its Analogs.*^{1,1)}

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Lenthionine, an odorous substance, has been isolated from *Lentinus edodes* (Berk.) Sing and the structure was established as 1,2,3,5,6-pentathiepane (I). 1,2,4,6-Tetrathiepane (II) and 1,2,3,4,5,6-hexathiepane (III) were also isolated from the mushroom in minor quantities. All these cyclic methylene polysulfides were synthesized from simple starting materials.

A precursor of lenthionine was isolated in crystalline form and the structure was deduced mainly on the basis of mass spectral studies. A possible mechanism for the formation of lenthionine and its analogs from the precursor was proposed.

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In the previous communication¹⁾ the isolation, structure, and synthesis of lenthionine, 1,2,3,5,6-pentathiepane (I), together with some discussions on the mass spectrum of the compound were described.

The present paper deals with the further isolation of the minor constituents from the mushroom and their structures defined as 1,2,4,6-tetrathiepane (II) and 1,2,3,4,5,6-hexathiepane (III), respectively. An improved synthesis of these compounds has been found. Somewhat surprisingly, a precursor of lenthionine (I) and 1,2,4,6-tetrathiepane (II) has been isolated in crystalline form.

The conventional and high resolutional mass spectral studies together with other physico-chemical measurements finally led to the assignment of the structure of this compound and also to the speculation of a possible mechanism for the formation of lenthionine (I) and 1,2,4,6-tetrathiepane (II) from the precursor.

Isolation and Structure of Lenthionine (I) and Its Analogs (II and III)

Dried mushrooms (5 kg.) were immersed in water overnight; during this period a characteristic odor of the mushroom evolved. The mushrooms were centrifuged and the wet cake was vigorously stirred with a mechanical stirrer in chloroform. The organic layer was separated and the solvent was evaporated under reduced pressure to yield a light yellow oil.

Careful chromatography of this oily material on silica gel with chloroform afforded six fractions. The least polar fraction, which was eluted first from the column and weighed 2.2 g., was rechromatographed on silica gel with *n*-hexane as solvent to permit separation of the following three fractions designated fraction-Ia, -Ib and -Ic, respectively.

From fraction-Ib was obtained lenthionine (I), which after recrystallization from dioxane melted at 60~61° and had a characteristic odor of the mushroom. The constituent from the fraction-Ic was purified by sublimation to give beautiful colorless prisms which melted at 79°. The mass spectrum was highly informative on the structure of this compound; as can be seen in Fig. 1b, a mass peak 60 is clearly visualized and this is ascribed to $-\text{CH}_2-\text{S}-\text{CH}_2-$ fragment, which was virtually absent in the spectrum¹⁾ of lenthionine (Fig. 1a).

*¹ Part of this study was presented at the 4th International Symposium on the Chemistry of Natural Products, IUPAC, Stockholm, June 26~July 2, 1966.

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1) Previous communication, K. Morita, S. Kobayashi: Tetrahedron Letters, 573 (1966).

These data together with the nuclear magnetic resonance (NMR) spectrum and elementary analysis led us to assign the structure (II) to this compound. The compound, however, was not a new one but had been synthesized by Baumann*³ in 1890. Our natural specimen showed an identical melting point with the recorded value.

The final product which was isolated from the fraction-Ia in an extremely low yield was pale yellow crystals, which showed no distinct melting point. The structure was established by the mass spectrum (Fig. 1c), which unambiguously demonstrated that the compound was 1,2,3,4,5,6-hexathiepane (III).

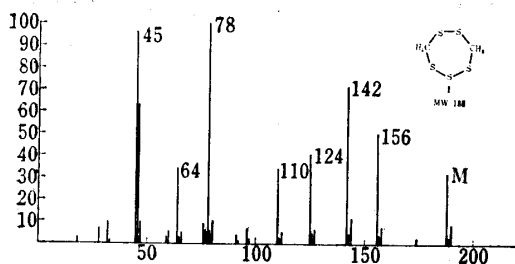


Fig. 1a. Mass Spectrum of Lenthionine (I)

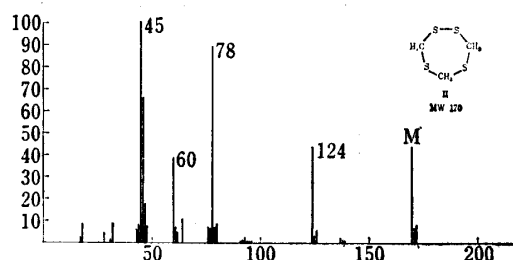


Fig. 1b. Mass Spectrum of 1,2,4,6-Tetrathiepane (II)

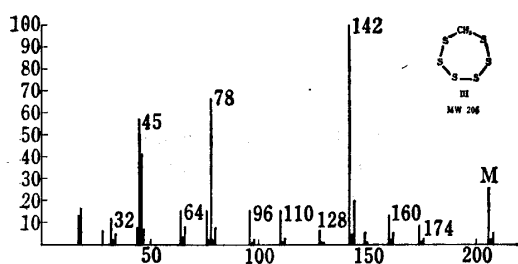


Fig. 1c. Mass Spectrum of 1,2,3,4,5,6-Hexathiepane (III)

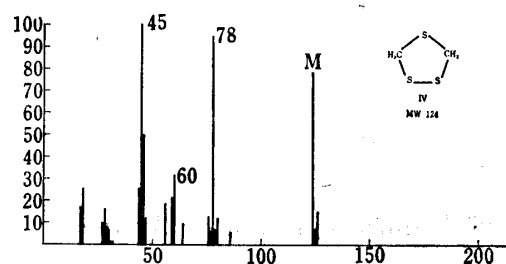


Fig. 1d. Mass Spectrum of 1,2,4-Trithiolane (IV)

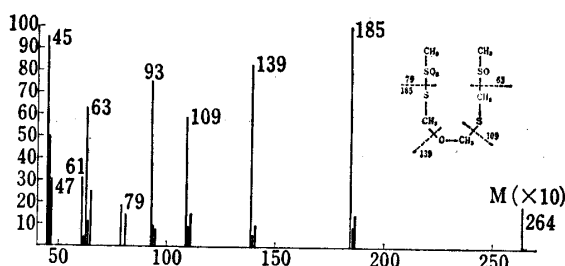


Fig. 1e. Mass Spectrum of SE-3

Improved Synthesis of Lenthionine and Its Analogs

A large quantity of lenthionine was required at this stage for certain biological tests and an attempt was made to find an improved synthesis of the compound. Finally, we came up with a facile synthesis of lenthionine and its analogs, which is summarized as follows: A solution of sodium polysulfide was adjusted to pH 8; to this was added a large excess of methylene chloride and the mixture was vigorously stirred at room temperature for several hours. The organic layer was separated, washed with water, dried over sodium sulfate and the solvent evaporated *in vacuo*. After

*³ E. Baumann: Ber., 23, 1869 (1890).

being left standing in a refrigerator colorless prisms of lenthionine separated in good yield.

From the mother liquor, after chromatography, was isolated the compound III which showed a complete identity with a natural specimen from the mushroom.

When the reaction was carried out at pH 12, no practical quantity of lenthionine was obtained. Instead, a good yield of a pale yellow low boiling liquid was obtained together with the compound II. This low boiling liquid failed to crystallize, however, the TLC*⁴ gave a single spot.

From an inspection of the mass spectrum (Fig. 1d) the structure IV was assigned to the compound. This structure might be regarded as the thio-ozonide of ethylene.

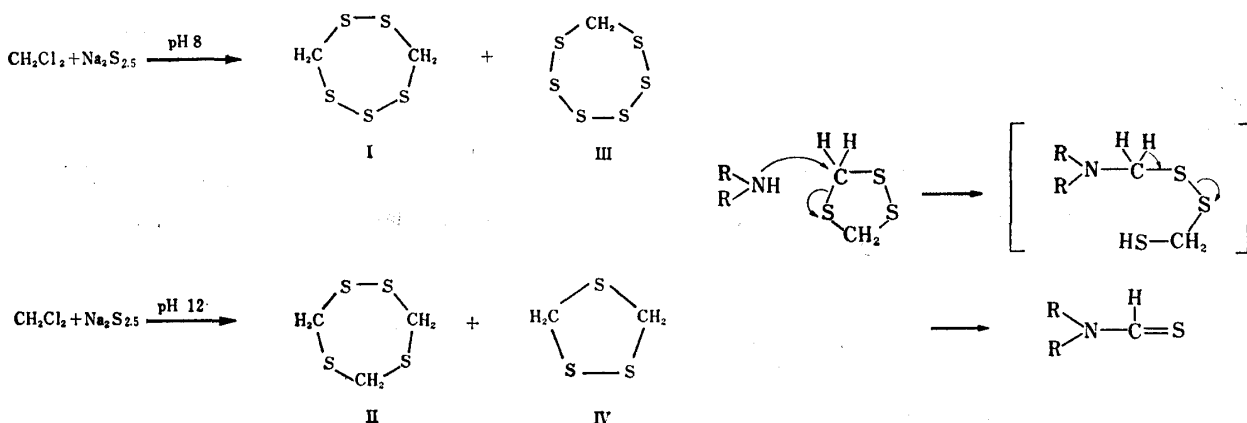


Chart 1. Improved Syntheses of Lenthionine and Its Analogs

Chart 2. Proposed Mechanism for the Thioformylation of Amines

A Novel Thioformylation of Amines

1,2,4-Trithiolane (IV) was produced from simple starting materials and was obtained in pure liquid by distillation under reduced pressure (b.p. 78~79°/3 mmHg). Interestingly, the compound when heated with a primary or secondary amine afforded the corresponding thioformamide as the sole product. The mechanism of this novel thioformylation reaction would be explained as in Chart 2.

Isolation and Structure of the Precursor (SE-3)

The precursor to lenthionine (I) and 1,2,4,6-tetrathiepane (II) was last obtained in crystalline form after careful chromatography of the crude chloroform extract from the mushroom. This compound, tentatively designated SE-3, was isolated from the fraction III and obviously was more polar than lenthionine in TLC.

By the studies with TLC, it was demonstrated that the compound SE-3 was a non-enzymic precursor of lenthionine (I) and 1,2,4,6-tetrathiepane (II), because the compound (SE-3) dissolved in organic solvents gradually decomposed to give the latter two compounds. It should be mentioned, however, that the compound SE-3 was not found as such in the dried mushroom, because it became extractable by organic solvents only after the dried mushrooms had been immersed in water. This implies that SE-3 must have arisen from a still unknown precursor.

The IR spectrum of the compound SE-3 showed strong absorption bands at 1295, 1102, 977, 962, and 953 cm^{-1} . A set of the former two is characteristic of a sulfone group and the latter three bands are ascribable to a sulfoxide.

*⁴ Thin-layer chromatography. Silica gel G was used and the micro plates were prepared by the spraying method (K. Morita, F. Haruta: J. Chromatog., 12, 412 (1963)). *n*-Hexane was used as a solvent and the spots were visualized in iodine vapor.

In the NMR spectrum a three-proton singlet at 2.50 p.p.m. was ascribed to a CH_3SO -group and the one at 3.05 p.p.m. to a CH_3SO_2 -group. Other three singlets were assigned to the methylene groups sandwiched in between sulfur and oxygen. These results led to the assignment of the structure SE-3 for the compound (Fig. 1e).

The above structural assignment receives further support from the conventional and high resolution mass spectral studies. As can be seen in Fig. 1e, the molecular peak and other main mass peaks are reasonably explained on the proposed structure.

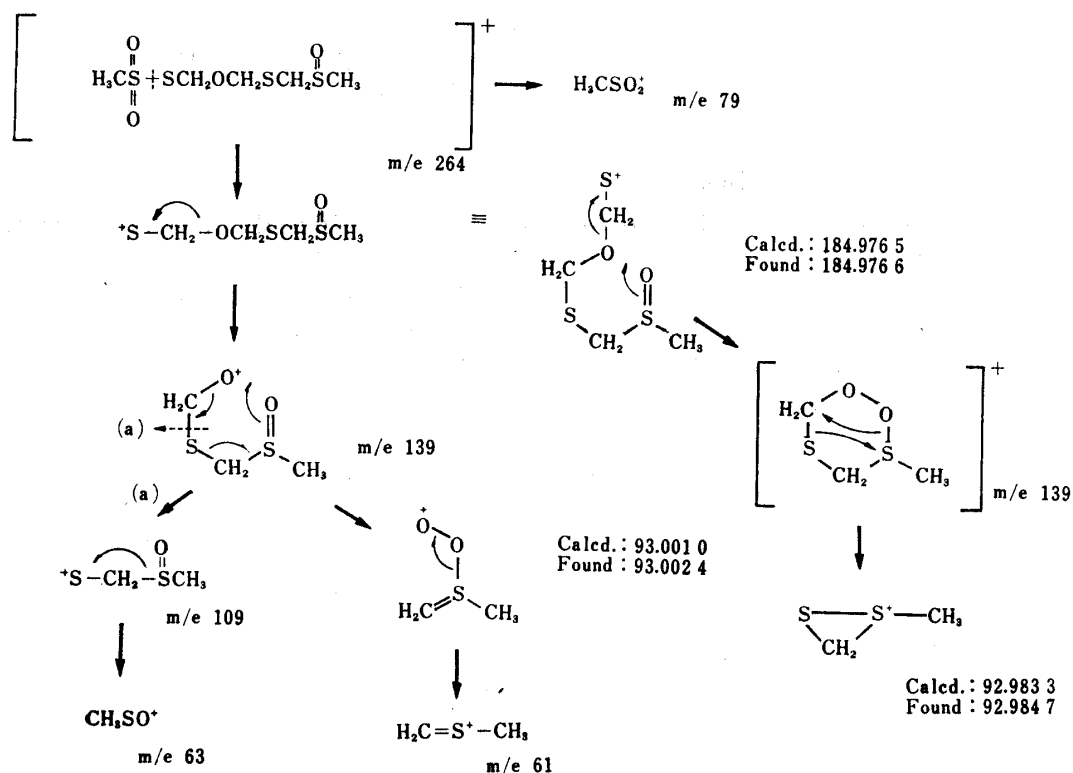


Chart 3. Proposed Structure and Mass Spectral Fragmentation of SE-3

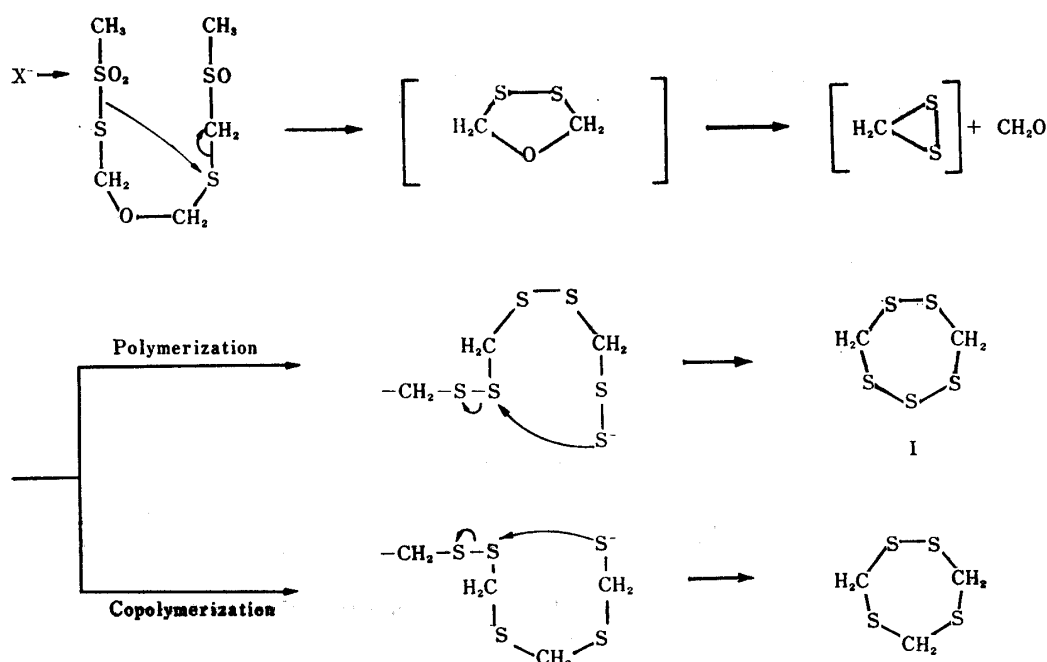


Chart 4. Proposed Mechanism for Synthesis of Lenthionine and Its Analogs

The high resolutional mass spectra were taken with the mass peaks 185 and 93. It was very interesting to note that the mass 93 was split into a doublet, *i.e.* 93.0024 and 92.9847. These two mass numbers are now explicable on the basis of the proposed mechanism of the fragmentation (Chart 3). Further this mechanism provides a good explanation for the mass peak 61.

A presumption on the mechanism of the formation of lenthionine and the compound (II) from the precursor SE-3 is summarized in Chart 4. When the precursor SE-3 is attacked by an anion, a five membered cyclic intermediate would first result, which in turn would give rise to methylene disulfide and formaldehyde. Polymerization of methylene disulfide followed by cyclization would finally give lenthionine. Similarly, copolymerization of methylene disulfide and formaldehyde with concomitant loss of water followed by cyclization should give the compound II.

Further Aspects of Lenthionine

It should be realized that the mushroom has been artificially grown on a commercial scale and is particularly prized as comestibles owing to its characteristic flavor. It is also worthy of note that lenthionine shows fairly strong antibiotic activities against a number of microorganisms including bacteria and fungi as shown in Table I. Further aspects which include the X-ray crystallographic analysis and the polarographic behaviors of the compound will be reported elsewhere.

TABLE I. Biological Activities of I and II

Test strain		MIC value : γ /ml.	
		I	II
<i>Bacillus subtilis</i> ^{a)} PCI 219	IFO 3513	50	250
<i>Staphylococcus aureus</i> ^{a)} 209 P	IFO 3061	250	250
<i>Escherichia coli</i> ^{a)}	IFO 3044	250	250
<i>Proteus vulgaris</i> ^{a)}	IFO 0337	50	250
<i>Piricularia oryzae</i> ^{b)}	IFO 5279	12.5	50
<i>Glomerella cingulata</i> ^{b)}	IFO 5925	12.5	125
<i>Trichophyton mentagrophytes</i> ^{b)}	IFO 5809	3.12	125
<i>Candida albicans</i> ^{b)}	IFO 0599	6.25	125
<i>Saccharomyces cerevisiae</i> ^{c)}	IFO 0209	6.25	—
<i>Cryptococcus neoformans</i> ^{b)}	IFO 0410	6.25	—
<i>Trichophyton rubrum</i> ^{b)}	IFO 5467	3.12	—

Agar dilution method.

a) Bouillon agar medium

meat ext. 1%

polypeptone 1%

NaCl 0.25%

agar 2.0%

pH 7.0

incubation : 37°, 24 hr.

b) Glucose bouillon agar medium

Bouillon agar medium + glucose 1%—pH 7.0,

incubation : 27°, 3 days.

c) Glucose bouillon agar medium—pH 7.0,

incubation : 27°, 24 hr.

Experimental

M.p. and b.p. are uncorrected. IR spectra were taken in KBr disk on a Hitachi EPI-S₂ infracord and NMR spectra on a Varian A-60 model in deuterochloroform with tetramethylsilane as the internal standard, unless otherwise stated.

Isolation of Lenthionine (I) and Its Analogs (II & III) from Dried Mushrooms—Dried mushrooms*⁵ (5 kg.) were immersed in water overnight. After centrifugation the wet cake was vigorously stirred in

*⁵ *Lentinus edodes* (Berk.) Sing.

chloroform to extract the odorous substance. The chloroform extract was evaporated under reduced pressure to yield a light yellow oil (23.3 g.). Chromatography of this oily material on silica gel*⁶ (4 kg.) with chloroform as solvent furnished the following six fractions: fraction-I (2.20 g.), fraction-II (4.37 g.), fraction-III (1.89 g.), fraction-IV (1.82 g.), fraction-V (3.14 g.), and fraction-VI (1.77 g.).

Fraction-I was rechromatographed on silica gel with *n*-hexane as solvent to yield the following three fractions, which were designated fraction-Ia, fraction-Ib, and fraction-Ic.

Fraction-Ib, a main constituent, was eluted after fraction-Ia and turned out to be lenthionine for the crystals obtained from this fraction after recrystallization from dioxane melted at 60~61° and showed no depression of the melting point on admixture with an authentic sample.¹⁾ Yield 560 mg. *Anal.* Calcd. for C₂H₄S₅: C, 12.75; H, 2.14; S, 85.11. Found: C, 12.89; H, 2.22; S, 85.13. From the fraction-Ic was obtained colorless prisms of 1,2,4,6-tetrathiepane (II), which melted at 79° and was readily purified either by sublimation or by recrystallization from dioxane; yield 300 mg.; NMR signals at 4.24 (4H, singlet) and 4.28 (2H, singlet) p.p.m. *Anal.* Calcd. for C₃H₆S₄: C, 21.18; H, 3.53; S, 75.29. Found: C, 21.26; H, 3.48; S, 75.03.

Fraction-Ia was evaporated *in vacuo* to afford a residue, which was dissolved in hot dioxane and left in a refrigerator to yield colorless prisms; m.p. 79°(sinter). Yield 7 mg.; NMR signal at 4.54 p.p.m. *Anal.* Calcd. for CH₂S₆: C, 5.82; H, 0.98; S, 93.20. Found: C, 6.37; H, 1.07; S, 92.46.

Synthesis of Lenthionine and 1,2,3,4,5,6-Hexathiepane (III)—A solution of Na₂S_{2.5} was prepared from Na₂S·9H₂O (300 g.) and sulfur (60 g.) in 1000 ml. of water, to which was bubbled a stream of hydrogen sulfide gas to adjust the pH of the solution at 8. The solution at this pH was covered with methylene chloride (1000 ml.) and the mixture was vigorously stirred at room temperature for several hours. The organic layer was separated, washed with water, dried over sodium sulfate and the solvent evaporated under reduced pressure to yield an oily material (40 g.). On standing at room temperature this oily material gradually went into a crystalline mass, which was separated from the liquid by centrifugation. Recrystallization from dioxane yielded colorless prisms of lenthionine; m.p. 60~61°. Yield 6.1 g.

A portion (4 g.) of the liquid substance was chromatographed on silica gel with *n*-hexane as solvent to yield a fraction, which run out before lenthionine. This fraction on evaporation furnished a crystalline residue which was recrystallized from dioxane to give pale yellow crystals; m.p. 79°(sinter). Yield 0.14 g. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1184, 815, and 723.

Synthesis of 1,2,4,6-Tetrathiepane (II) and 1,2,4-Trithiolane (IV)—A solution of Na₂S_{2.5} (pH 12.7), which was prepared from Na₂S·9H₂O (300 mg.) and sulfur (60 g.) in 1000 ml. of water, was covered with methylene chloride (1000 ml.) and the mixture was vigorously stirred at room temperature for seven hours. During this period the pH of the solution shifted to 11.9. The organic layer was separated, washed with water, dried over sodium sulfate and the solvent was evaporated under reduced pressure to yield an oily material (34.9 g.). Upon distillation under reduced pressure a pale yellow liquid of 1,2,4-trithiolane (IV) was obtained; b.p. 78~79°. Yield 13.3 g.; NMR signal at 4.22 p.p.m. *Anal.* Calcd. for C₂H₄S₃: C, 19.34; H, 3.25; S, 77.41. Found: C, 19.86; H, 3.11; S, 76.24. The residue in the flask solidified to a crystalline mass, which was recrystallized from dioxane to yield 1,2,4,6-tetrathiepane (II). Yield 7.6 g. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1364, 1220, 1198, 867, 730, and 702.

Thioformylation of Amines with 1,2,4-Trithiolane (IV)—General procedure: Primary or secondary amine was mixed with 1,2,4-trithiolane (IV) and the mixture was heated on a steam bath (60~70°) for 30 min., during this period the amine dissolved into the solution to give a viscous solution. The thioformylamine was isolated from the crude reaction mixture by chromatography.

Thus from morpholine (0.87 g.) was obtained N-thioformylmorpholine, which melted at 67° after recrystallization from methanol. Yield 0.94 g. *Anal.* Calcd. for C₅H₉ONS: C, 45.79; H, 6.92; N, 10.68; S, 24.42. Found: C, 45.79; H, 6.86; N, 10.60; S, 24.87.

Similarly, from piperidine (0.85 g.) was obtained N-thioformylpiperidine. Yield 0.28 g. *Anal.* Calcd. for C₆H₁₁NS: C, 55.78; H, 8.58; N, 10.85; S, 24.78. Found: C, 55.52; H, 8.36; N, 10.65; S, 24.88.

Isolation of SE-3—The fraction-III (1.89 g.) was twice rechromatographed on silica gel with benzene-ethyl acetate (30:1) as solvent. A crystalline residue, which was obtained as a main product from this fraction, was recrystallized from acetone to yield colorless needles; m.p. 80~82°. Yield 20 mg. NMR signals (CDCl₃) at 2.50 (3H, singlet), 3.05 (3H, s), 3.97 (2H, s), 4.05 (2H, s), and 4.19 (2H, s) p.p.m. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 2990, 1295, 1102, 997, 963, 953, and 784.

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*⁶ E. Merck, A. G., under 0.08 mm.