

### S-Allylmercapto-L-cysteine

On elution with 0.2M ammonium formate buffer (pH 3.97) the new sulfur-containing amino acid emerged after fraction of  $\gamma$ -L-glutamyl-S-allylmercapto-L-cysteine. The desalted fraction was evaporated to dryness *in vacuo* and the residue was recrystallized from hydrous acetone and 10 mg. of scales, m.p. 187~188°(decomp.),  $[\alpha]_D^{25.5} -107.9 \pm 5^\circ$  ( $c=0.4$ , 6N HCl) was obtained from 5 kg. of garlic bulbs.  $R_f$  0.80(PhOH-0.08% NH<sub>4</sub>OH=4:1); 0.60(BuOH-AcOH-H<sub>2</sub>O=5:1:4). On the paper chromatogram, it showed positive with iodoplatinate reagent and also showed positive with cyanide and nitroprusside reactions.  $R_f$  values and infrared spectrum of this amino acid were quite identical to those of authentic sample of S-allylmercapto-L-cysteine. From these facts, the new amino acid isolated was confirmed to be S-allylmercapto-L-cysteine. Cavallito, *et al.*<sup>3)</sup> reported that S-allylmercapto-L-cysteine is synthesized by the reaction between cysteine and allicin (diallylthiosulfinate) which is formed from alliin (S-allyl-L-cysteine sulfoxide) in the presence of alliinase.

In the present experiment, the authors have isolated S-allylmercapto-L-cysteine from the garlic bulbs which were heated to destroy the alliinase activity. From this fact, it is probable that the presence of S-allylmercapto-L-cysteine is not an artifact during the isolation procedure. This consideration was further supported by the following observations: The garlic bulbs in the frozen state were cut into small pieces and then extracted with 80% methanol. From the extract, S-allylmercapto-L-cysteine and  $\gamma$ -L-glutamyl-S-allylmercapto-L-cysteine were obtained. The extraction was also carried out with 80% methanol containing excess of formaldehyde, in order to prevent the reaction of cysteine with allicin during extraction procedure. Even under such conditions, the above two cysteine derivatives were obtained with the similar yields.

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### New Sulfur containing Amino Acids in Cabbage: Isolation and Identification of L-Homomethionine (L-5-Methylthionorvaline)

Tracer technique was used for the detection of sulfur-containing amino acids in cabbage and an excised aerial part of cabbage (275 g.) was cultivated in 5 ml. of water containing 1 mc. of carrier free H<sub>2</sub><sup>35</sup>SO<sub>4</sub>. After all of the solution was absorbed into the plant, the plant was cultivated with a SO<sub>4</sub><sup>2-</sup> free hydroponic solution for 4 days. The plant was homogenized and extracted with 80% methanol. The soluble fraction was passed through a column of Amberlite IR-120 (H<sup>+</sup> from) and the adsorbed amino acids were eluted with 4% ammonium hydroxide solution. The total radioactivity in this eluate, which contained amino acids and peptides, was 91.5  $\mu$ c. Yield, 1.71 g. A part of

the eluate was subjected to two dimensional paper chromatography and the remainder was applied on a column (3.5×74 cm.) of Dowex 50×4 equilibrated with 0.05M ammonium formate buffer at pH 2.50. The chromatography was run by gradient elution according to the method of Thompson.<sup>1)</sup> The radioactivity was plotted against the tube numbers, and each peak having radioactivity was pooled, lyophilized, desalting, and examined by paper chromatography. At least 14 kinds of <sup>35</sup>S-labeled amino acids and peptides were detected by radioautography and by color reaction with ninhydrin and iodoplatinate reagents.

Among these spots cystine, cysteic acid, S-methylcysteine, S-methylcysteine sulfoxide,  $\gamma$ -glutamyl-S-methylcysteine, methionine and S-methylmethionine could be identified by cochromatography with the authentic samples. The highest labeling spot was found as S-methylcysteine sulfoxide (total radioactivity: 23.1  $\mu$ c.), which is the major sulfur-containing amino acid in cabbage.

Among the <sup>35</sup>S-labeled amino acids, the authors were aware of a presence of methionine-like amino acid. From a Dowex 50 column it emerged just after methionine and leucine. It moved faster than methionine by the paper chromatography with BuOH-AcOH-H<sub>2</sub>O (4:1:1) and moved at the same speed as methionine with PhOH-H<sub>2</sub>O (4:1).

Electrophoretic mobility of this amino acid (30 v./cm.) at pH 6.5 (pyridine-AcOH-H<sub>2</sub>O = 10:0.4:90) was identical to that of methionine. The product oxidized with hydrogen peroxide moved at the same speed with methionine sulfoxide by the paper chromatography in the above solvent systems. In the air, the unknown compound was easily oxidized just like methionine.

To isolate this unknown compound in large scales, 20 kg. of cabbage was treated in a manner similar as described above. The isolated unknown amino acid fraction was contaminated with an amino acid having Rf 0.10 with a solvent system of BuOH-AcOH-H<sub>2</sub>O.

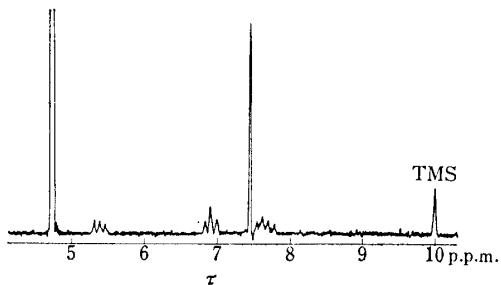


Fig. 1. The Nuclear Magnetic Resonance Spectrum of Isolated Amino Acid in Deuterium Oxide

This contaminant was removed by a cellulose powder column chromatography in a solvent system of BuOH-AcOH-H<sub>2</sub>O. After crystallization from hydrous ethanol, 50 mg. of fine scales, m.p. 223~225°(decomp.),  $[\alpha]_D^{25.5} +21.0$  ( $c=0.30$ , 6N HCl) was obtained. The elementary composition of this amino acid corresponded to the formula C<sub>6</sub>H<sub>13</sub>O<sub>2</sub>NS (Calcd: C, 44.16; H, 8.03; N, 8.58. Found: C, 44.39; H, 8.10; N, 8.74). This amino acid was positive with iodoplatinate and ninhydrin reagents, negative with cyanide and nitroprusside reagents on the paper, and gave norvaline by the desulfurization with Raney nickel.

The nuclear magnetic resonance spectrum of the amino acid showed a presence of CH<sub>3</sub>S- at 7.43  $\tau$  (Fig. 1).

From these results, the structure of the amino acid isolated was considered to be L-homomethionine (L-5-methylthionorvaline) CH<sub>3</sub>-S-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH(NH<sub>2</sub>)-COOH. Such a presumption was finally proved by comparing the Rf values (0.85: PhOH-H<sub>2</sub>O = 4:1, 0.49: BuOH-AcOH-H<sub>2</sub>O = 4:1:1) and nuclear magnetic resonance spectrum with those of the authentic sample of DL-homomethionine which was synthesized by the method of Kjaer and Wagner.<sup>2)</sup> Furthermore, the Rf values of a product obtained with hydrogen peroxide

1) A. R. Thompson: Biochem. J., 61, 253 (1955).

2) A. Kjaer, S. Wagner: Acta Chem. Scand., 9, 721 (1955).

agreed with those of homomethionine sulfoxide. This is the first report on the isolation of L-homomethionine as natural product.

Studies on the biosynthesis of L-homomethionine are now in progress, and it was found that thiomethyl group of L-homomethionine is arisen from methionine. It is probable that L-homomethionine is biosynthesized from  $\alpha$ -amino- $\delta$ -hydroxyvaleric acid and methyl mercaptan which is derived from methionine. Recently,  $\alpha$ -amino- $\delta$ -hydroxyvaleric acid has been isolated by Thompson, *et al.*<sup>3)</sup> from higher plant.

The details of these experiments will be published later.

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3) J. F. Thompson, C. J. Morris, G. E. Hunt : *J. Biol. Chem.*, **239**, 1122 (1964).

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### Structure of Digiprolactone

In the preceding communication,<sup>1)</sup> the structure of digiprolactone (I), m.p. 149/151°,  $C_{11}H_{16}O_3$ , was postulated as I. This report describes confirmatory evidences for this structure.

In order to confirm the structure of the cyclohexane moiety of I, the following degradative reactions were carried out.

Digiprolactone acetate (II) was treated with ozone at -80°, followed by reductive fission of ozonide to give an oxoester (III),<sup>2)</sup> which was hydrolyzed with sodium carbonate without purification to afford a dihydroxyketone (IV), m.p. 119°,  $C_9H_{16}O_3$ ,  $[\alpha]_D^{24} +22.2$  (c=1.067,  $CHCl_3$ ), UV :  $\lambda_{max}^{95\%EtOH}$  289 m $\mu$  (log  $\varepsilon$  1.65), IR  $\lambda_{max}^{Nujol}$   $\mu$  : 2.90, 3.08 (OH); 5.88 (C=O). Oxidation of IV with chromic anhydride gave a hydroxydione (V), m.p. 72~72.3°,  $C_9H_{14}O_3$ ,  $[\alpha]_D^{24\cdot5} -13.1$  (c=1.063,  $CHCl_3$ ), IR  $\lambda_{max}^{Nujol}$   $\mu$  : 3.00 (OH); 5.88 (C=O), which was converted with dil. sulfuric acid to an enedione (VI), UV :  $\lambda_{max}^{95\%EtOH}$  238 m $\mu$ , IR  $\lambda_{max}^{CCl_4}$   $\mu$  : 5.94 (C=O); 6.16 (C=C). Reduction of VI with zinc in acetic acid gave a diketone (VII), m.p. 66°, IR :  $\lambda_{max}^{CCl_4}$  5.82  $\mu$  (C=O), which was identified with the authentic sample of 2,6,6-trimethyl-1,4-cyclohexanenedione, by mixed melting point determination and comparison of infrared spectra.

The authentic sample of VII was synthesized from isophorone (VIII) by its conversion to hydroxyisophorone (IX),<sup>3,4)</sup> followed by the double bond isomerisation with *p*-toluenesulfonic acid to VII. Oxidation of IX with chromic anhydride afforded VI which also gave VII by reduction with zinc in acetic acid.

- 1) T. Wada, D. Satoh : *This Bulletin*, **12**, 752 (1964).
- 2) J. v. Euw, T. Reichstein : *Helv. Chim. Acta*, **47**, 711 (1964).
- 3) M. S. Kharasch, E. K. Fields : *J. Am. Chem. Soc.*, **63**, 2308 (1941).
- 4) O. Isler, *et al.* : *Helv. Chim. Acta*, **39**, 2041 (1956).