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Biosynthesis of S-Methyl-L-cysteine and S-Methyl-L-cysteine Sulfoxide from Methionine in Garlic

S-methyl-L-cysteine and its sulfoxide are widespread occurred in plants. Recently, Arnold and Thompson[1] have shown that S-methyl-L-cysteine sulfoxide is formed from biological oxidation of S-methyl-L-cysteine in crucifers. Although Wolf, *et al.*[2] have found that S-methyl-L-cysteine is enzymatically synthesized in an extract of yeast from methyl mercaptan and L-serine, the biogenesis of S-methyl-L-cysteine in higher plants is obscure. It has been known that methyl mercaptan can be arisen in microorganisms[3] and in the rat[4] from methionine. Evidence has now been obtained for the formation of S-methyl-L-cysteine and its sulfoxide from methionine in garlic plant. If methyl mercaptan is formed from methionine in higher plants, it is considered that S-methyl-L-cysteine and its sulfoxide may be derived from methionine.

An exiced aerial part of garlic plant (17 g.) was cultivated in 3 cc of water containing 8×10^5 c.p.m. L-methionine [^{35}S] (10 mg.). After 5 hr. all of the solution was taken up by the plant, then it was dipped in water, and the cultivation was continued for 24 hr. The plant was boiled in water for 10 min. After cooling, it was homogenized with the same water that used for boiling. The pH of homogenate was adjusted to 4.0 with AcOH and was centrifugated. The water soluble fraction was put through a column of Amberlite IR 120 (H^+ form) and the adsorbed amino acids were eluted with 4% NH_4OH solution. The total radioactivity of the amino acids was 15.3×10^4 c.p.m. A part of the amino acids (3×10^3 c.p.m.) was subjected to two dimentional paper chromatography with the solvent systems of (a) phenol:0.08% NH_4OH (4:1), (b) BuOH:AcOH:H_2O (4:1:2). On radioautogram four spots were detected and their Rf values were just the same as those of authentic samples of S-methyl-L-cysteine sulfoxide [(a) 0.65, (b) 0.18], methionine sulfoxide [(a) 0.78, (b) 0.23], S-methyl-L-cysteine [(a) 0.75, (b) 0.40] and methionine [(a) 0.80, (b) 0.52]. The remaining amino acids (15×10^4 c.p.m.) were adsorbed on a column (2×70 cm.) of Dowex 50X4 buffered at pH 2.40 with 0.05*M* $HCOONH_4$ buffer. The development of adsorbed amino acids was started using pH 2.5 $HCOONH_4$ buffer and the pH of the buffer was gradually risen to 3.5. The flow rate of developer was 10 cc. per 0.5 hr. An elution curve was obtained by plotting the radioactivity* against the tube

*1 Radioactivity assays were carried out with a GM-tube with a thin mica window. Samples were counted at an infinite thinness.

1) W. N. Arnold, J. F. Thompson: Biochem. Biophys. Acta, **57**, 604 (1962).
2) E. C. Wolff, S. Black, P. F. Downey: J. Am. Chem. Soc., **78**, 3958 (1956).
3) F. Challenger, P. T. Charlton: J. Chem. Soc., **1947**. 424
4) E. S. Canellakis, H. Tarner: Arch. Biochem. Biophys. **42**, 387 (1953).

number. Four sharply defined peaks were obtained. The peaks were pooled, lyophilized and desalted. As for the residue obtained, two dimentional paper chromatography with solvent systems (a) and (b) was carried out and the radioactive spots were ascertained by radioautography, and then identified by cochromatography using authentic samples. From these results, it was found that cycloalliin existed in a peak 1 separated from S-methyl-L-cysteine sulfoxide and alliin in a peak 2 separated from methionine sulfoxide, respectively. Cycloalliin and alliin were present to a much greater extent than any other amino acids in a garlic, but these amino acids did not have radioactivity. (Fig. 1, Table I).

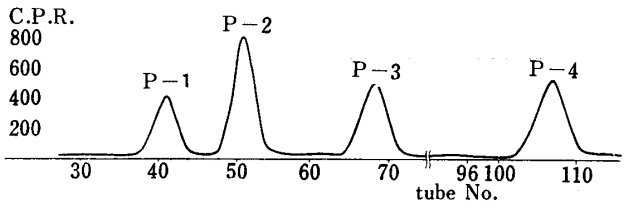

Fig. 1. Separation of ^{35}S-Labeled Amino Acids

Fraction of 10 cc. per 30 min. were collected and 0.05 cc. of sample in each fraction was counted.

TABLE I. Radioactivity of Each Fraction

Peak No.		Tube No.	Total Radioactivity (c. p. m.)	Recovery (%)
p-1	(S-Methyl-L-cysteine sulfoxide fraction)	39～43	2.0×10^4	13.3
p-2	(Methionine sulfoxide fraction)	48～53	3.6×10^4	24.0
p-3	(S-Methyl-L-cysteine fraction)	65～71	3.1×10^4	20.6
p-4	(Methionine fraction)	103～110	3.3×10^4	22.0
Total Recovery (1+2+3+4)			12.0×10^4	79.9

The results obtained were further confirmed by the following manner. S-methyl-L-cysteine sulfoxide (p-1) was reduced to corresponding sulfide with HI and S-methyl-L-cysteine (p-3) was oxidized to sulfoxide with H_2O_2, respectively. There were no other radioactive amino acids except above four compounds in this experiment. These facts show that the S-methyl group of S-methyl-L-cysteine and its sulfoxide is arisen from methionine.

Above results will be confirmed and extended in the experiments using methionine-[methyl^{14}-C].

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