

**The Detection, Isolation, and Identification of  $\gamma$ -Glutamyl-S-Methylcysteine from Beans<sup>1</sup>**

The isolation and characterization of S-methyl-L-cysteine from the nonprotein nitrogen fraction of the kidney bean has recently been reported by Thompson *et al.* (1). At that time, the authors were aware of the presence of this compound in the form of a peptide in the same fraction of the kidney bean. The peptide has now been isolated from ion exchange resin columns and further purified through separation on a cellulose column followed by ion exchange chromatography on Dowex-1.

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<sup>1</sup> Presented before the Division of Biological Chemistry at the 132nd meeting of the American Chemical Society, New York, N. Y., September 9, 1957.

The newly isolated peptide gave positive iodoplatinate, ninhydrin, and Rydon-Smith tests but was not found to contain S—S or —SH groups as shown by negative nitroprusside and cyanide reactions. Acid hydrolysis yielded two amino acid fragments which were identified by quantitative paper chromatography to be a 1:1 mole ratio of glutamic acid and S-methylcysteine. Preparation of the dinitrophenyl derivative and subsequent hydrolysis revealed glutamic acid as the N-terminal compound.

Synthetic  $\gamma$ -glutamyl-S-methyl-L-cysteine was prepared from glutathione and its infrared curve found to agree in detail with the natural peptide. On prolonged standing or exposure to the acid resin column some  $\gamma$ -glutamyl-S-methylcysteine sulfoxide formed as was evidenced from the appearance of a small but characteristic sulfoxide peak at  $1020\text{ cm}^{-1}$ .

The isolated substance was found to be indistinguishable from the synthetic peptide on paper chromatograms employing water-saturated phenol ( $R_f$  0.47) and *n*-butanol-acetic acid-water ( $R_f$  0.18; based on 0.27 for S-methylcysteine). On treatment of the natural peptide with ninhydrin it yielded 95% of the theoretical  $\text{CO}_2$  for a single free amino and  $\alpha$ -carboxyl group (2) (compared to 96% for leucine), thus establishing the  $\gamma$ -glutamyl linkage. Furthermore, the ratio of carboxyl nitrogen to total nitrogen established the fact that the compound is a dipeptide inasmuch as only one structure might be written to fit the above facts.

This evidence in conjunction with an elemental analysis proves that the naturally occurring peptide is  $\gamma$ -glutamyl-S-methylcysteine. Very similar optical rotations  $[\alpha]_D^{20} - 25.1^\circ$  ( $\text{H}_2\text{O}$ ,  $c = 2.87$ ) for the synthetic and the isolated compound would further suggest that the peptide is  $\gamma$ -L-glutamyl-S-methyl-L-cysteine.

$\gamma$ -Glutamyl-S-methylcysteine was converted to  $\gamma$ -glutamyl-S-methylcysteine sulfoxide with hydrogen peroxide (3). The latter compound has  $R_f$  values of 0.39 and 0.18 in the phenol and acid butanol solvents, respectively. Paper chromatography of the nonprotein nitrogen fraction of the bean shows the presence of smaller amounts of the sulfoxide of  $\gamma$ -glutamyl-S-methylcysteine. However, the presence of this compound might well be an artifact resulting during the chromatographic separation in view of the fact that pure (as determined by infrared) synthetic  $\gamma$ -L-glutamyl-S-methyl-L-cysteine yields about 10% of the peptide sulfoxide on the paper chromatogram.

$\gamma$ -Glutamyl-S-methylcysteine is present to the extent of approximately one-third of the nonprotein nitrogen amino of the mature kidney bean, and snap bean and its presence in other leguminous seeds might well be anticipated. That this peptide might be a storage form of methyl groups and  $\gamma$ -glutamylcysteine, the direct precursor of glutathione in bean seedlings (4), is an interesting possibility. This and other possible significant roles of the peptide are being further investigated.

More complete details are to be published later.

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