Isolation of (−) S-Methyl-L-Cysteine from Beans (*Phaseolus vulgaris*)

THE recent isolation[1,2] of S-methyl-L-cysteine sulphoxide from two crucifers, turnips and cabbage, and the chromatographic evidence[3] for its occurrence in many related plants suggested the possibility that methyl cysteine should also occur in plants. In addition, preliminary evidence has clearly indicated that the turnip can readily form the sulphoxide from methyl cysteine. A careful examination[3] of the protein and non-protein fractions of several crucifers revealed no methyl cysteine. Previous work[4] on the non-protein amino-acids of bean seeds showed a ninhydrin-reactive spot which gave an iodoplatinate test[5] indicative of the sulphur amino-acids. This material was found on a two-directional chromatogram (phenol : butanol–acetic acid) in a position corresponding to that of methyl cysteine and close to that of γ-amino-butyric acid. This compound has been isolated and identified as (−) S-methyl-L-cysteine.

Dry kidney bean seeds were ground and extracted with 50 per cent alcohol. The extract was treated with mercuric acetate, and the resultant precipitate was separated and decomposed with hydrogen sulphide. After removal of mercuric sulphide, the solution was passed through a column of sulphonic acid ion exchange resin ('Dowex 50-X4') in the acid form and the column was washed. The amino-acids which were retained by the resin were fractionated by displacement chromatography with 0·10 *N* ammonia[6]. The fractions containing the unknown material were contaminated with glutamic acid and asparagine. These three compounds were separated by chromatography on a paper roll[7] using 70 per cent ethanol. The unknown material was purified by three crystallizations from 95 per cent ethanol. Approximately 150 mgm. were obtained from 5 kgm. of beans. The isolate was found to be identical with synthetic (−) S-methyl-L-cysteine with respect to infra-red spectra, optical rotation $[\alpha]_D^{25°} = -26°$ ($c = 2·5$), decomposition point (about 220° C.), and paper chromatography in phenol, butanol–acetic acid and collidine–lutidine ($R_F = 0·71$, 0·27 and 0·38, respectively). The elementary analysis of the isolate was C, 35·6; H, 7·0; N, 10·2; S, 23·5. The calculated values are C, 35·6; H, 6·67; N, 10·37; S, 23·7.

The presence of methyl cysteine in legumes and its sulphoxide in crucifers suggests that these two related compounds may have a widespread occurrence in plants. This possibility, coupled with the preliminary evidence of their interconversion, may foreshadow an interesting metabolic role for the methyl cysteine–methyl cysteine sulphoxide system in plants.

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Malate Synthesis in a Cell-free Extract from a Crassulacean Plant

EXTRACTS of spinach leaves[1] and of wheat germ[2] contain a phosphoenolpyruvic carboxylase which, in the presence of magnesium or manganese ions, catalyses the reaction:

$$\text{phosphoenolypyruvate} + CO_2 \rightarrow \text{oxalacetate} + \text{orthophosphate} \quad (1)$$

In the presence of reduced diphosphopyridine nucleotide and malic dehydrogenase, the oxalacetate is reduced to malate;

$$\text{oxalacetate} + \text{DPNH} \rightarrow \text{malate} + \text{DPN} \quad (2)$$

The linked reaction,

$$\text{phosphoenolpyruvate} + CO_2 + \text{DPNH} \rightarrow \text{malate} + \text{DPN} + \text{orthophosphate} \quad (3)$$

may then be followed spectrophotometrically by measuring the decrease in absorption at 340μ caused by the oxidation of reduced diphosphopyridine nucleotide.

It has been suggested that phosphoenolpyruvate carboxylase may function in the synthetic accumulation of dicarboxylic acids in plant tissues and especially in those of the Crassulaceae[2,3]. This communication reports that the leaves of *Kalanchoë crenata* and two other Crassulacean plants contain this enzyme. Extracts of *Kalanchoë* leaves known to contain malic dehydrogenase brought about a rapid oxidation of reduced diphosphopyridine nucleotide in the presence of phosphoenolpyruvate and magnesium or manganese ions. Phosphoenolpyruvate could not be replaced by pyruvate and adenosine triphosphate. There was no appreciable decrease in absorption when reduced triphosphopyridine nucleotide was substituted for reduced diphosphopyridine nucleotide.

Pre-incubation of phosphoenolpyruvate with the leaf extract and magnesium ions caused increases in the initial rate of oxidation of added reduced diphosphopyridine nucleotide. A two-stage reaction is implied by the fact that, within limits, the initial rate of oxidation was directly related to the length of the pre-incubation period.

Production of malate during the course of the linked reaction (3) has been shown chromatographically and the hydrazone of oxalacetic acid prepared, and separated, from reaction mixtures containing no reduced diphosphopyridine nucleotide. Labelled malate and labelled oxalacetic hydrazone have been similarly prepared by adding radioactive bicarbonate to the appropriate reaction mixture. This is believed to be the first demonstration of malate synthesis *in vitro* catalysed by a cell-free preparation from a Crassulacean plant.

Bandurski[3] has reported a stimulation of phosphoenolpyruvic carboxylase by carbon dioxide. This has been confirmed by Brown[4] and by myself, who found a linear relationship between the reciprocals of reaction-rate and the reciprocals of (low) concentrations of both phosphoenolpyruvate and carbon dioxide. Between 0·1 and 10 per cent the rate of the linked reaction was affected only slightly, rising gradually below 0·5 per cent and then declining. Above 10 per cent this decline became pronounced, and the reaction was almost completely inhibited in 70 per cent carbon dioxide (that is, in a solution containing the bicarbonate calculated from the Henderson Hasselbach equation[5] to be in equilibrium