

THE OCCURRENCE AND METABOLISM OF FREE AMINO ACIDS, IN SOME SELECTED
PLANTS OF SRI LANKA

A THESIS

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by

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ABSTRACT

This thesis deals with the occurrence and metabolism of free amino acids, in some selected plant genera of Sri Lanka. Techniques of column chromatography, paper partition chromatography, electrophoresis and autoradiography, were mainly employed in this study. The body of the thesis is divided into the following three Sections :

Section I - The occurrence and distribution of free amino acids of selected genera.

Section II - Metabolic studies

- a) Germination studies of Atylosia trinervia (Spreng.) Gamble and Dialium ovoideum Thw.
- b) Transamination studies of albizziine (α - amino - β - ureido propionic acid) with the keto acids, α - ketoglutaric acid and pyruvic acid.
- c) Radioactive feeding experiments of Dialium ovoideum Thw. seedlings.

Section III - Chemotaxonomic studies of the two Dicotyledonous families, Lythraceae and Melastomaceae.

In Section I, seeds belonging to thirty three species of plants were examined by paper chromatography, for the occurrence of free amino acids. The plant species studied can be broadly categorised into Leguminous and Non-Leguminous plants. The free amino acid patterns of most of these plant species are reported for the first time. However, for the purpose of identifying certain unusual amino acids, some plant species whose free amino acids have been reported previously, have been re-examined.

In addition to the usual 'protein' amino acids, the occurrence of twenty four 'non-protein' amino acids and six γ -glutamyl peptides, were shown in their free state. The presence of a number of other 'non-protein' ^{amino} acids too, were suspected. Most of these unidentified ninhydrin positive compounds occurred in insignificant concentrations. All these 'non-protein' amino acids and γ -glutamyl peptides have been identified and isolated previously. The occurrence of some of the 'non-protein' amino acids in plant species not recorded previously, is reported in this thesis for the first time, eg. the occurrence of the amino acid albizziine in the seeds of Dialium species. Some of these 'non-protein' amino acids were found to occur in very high concentrations, of the order of 2 to 3% of the dry weight of the seeds. No attempt was made however, to isolate any of these 'non-protein' amino acids on a large scale, as these have been previously reported. But an isolation of albizziine in a crude form was carried out on a small scale, in order to carry out the transamination experiments.

Section II deals with metabolic studies, and is sub-divided into germination studies, transamination studies, and studies on radioactive feeding experiments.

The germination studies relate to the fate of free amino acids and γ -glutamyl peptides, during the initial periods of growth of the two plant species, Atylosia trinervia (Spreng.) Gamble and Dialium ovoideum Thw. These studies were carried out with special reference to albizziine in Dialium ovoideum, and to the three γ -glutamyl peptides, γ -glutamyl phenylalanine, γ -glutamyl β -alanine and the unidentified peptide A-2, in Atylosia trinervia. In Dialium ovoideum, seven stages of germination, upto the stage of the disappearance of the cotyledons were studied. In addition, in Dialium ovoideum a stage of germination where the seedlings were etiolated was also studied.

In Atylosia trinervia three stages of germination upto the stage of the appearance of the first pair of leaves were studied. In Atylosia trinervia, it was observed, among other changes, that the three δ -glutamyl peptides, underwent metabolic changes independant of each other during germination. δ -Glutamyl phenylalanine showed a gradual decrease in concentration during germination, δ -glutamyl β -alanine showed a fluctuation in concentration (increase followed by a decrease in concentration), and A-2 showed no changes in concentration during germination. In Dialium ovoideum, among other changes, it was shown that the concentration of albizziine, remained more or less constant during germination. Another important observation made was the appearance of δ -methylene glutamine and δ -methylene glutamic acid during the initial stages of germination, and its gradual decrease and final disappearance during the last stages. The etiolated seedlings showed a marked difference in the free amino acid pattern from that of healthy seedlings. Most 'non-protein' amino acids present in the healthy seedlings were absent in the etiolated seedlings. The spot corresponding to albizziine on the paper chromatogram, which was present in unusually high concentration in the healthy seedlings, was completely masked by the brownish purple spot of asparagine in the etiolated seedlings.

As germination studies with Dialium ovoideum seedlings did not give much evidence as to the fate of albizziine in this plant, the transamination experiments were designed to investigate whether or not albizziine underwent transamination with the keto acids, α -keto-glutaric acid and pyruvic acid. The enzyme source for these experiments was obtained from two different plants, namely, Phaseolus aureus Roxb. and Dialium ovoideum Thw. It was shown that albizziine underwent transamination with α -ketoglutaric acid using the enzyme source from both the plants named above. The rate of transamination was however found to be rather low. Results of these experiments indicate that the transaminase involved in this transamination reaction is probably non-specific.

An attempt was made to determine the pathway for the breakdown of albizziine in Dialium ovoideum Thw., by radioactive feeding experiments. However, due to the non availability of the required radioactive material these experiments could not be carried out as planned. Experiments were carried out with the only available radioactive material, namely labelled monochloroacetic acid and thiourea. The results obtained were not considered significant.

Section III deals with chemotaxonomic studies of the two Dicotyledonous families, Lythraceae and Melastomaceae. An attempt was made to use the free 'non-protein' amino acids as a chemotaxon and to obtain a characteristic free amino acid pattern for each of the above named families. These two families were selected, because of the uncertain taxonomic position of Axinandra zeylanica Thw. According to various authors, there is a disparity in the classification of Axinandra zeylanica Thw. and it is placed differently in these two families. Seven species of the family Melastomaceae and four species of the Lythraceae, were examined by paper partition chromatography, for their free amino acids. Eight ninhydrin positive compounds (not present in appreciable concentrations) remained unidentified. The 'non-protein' amino acids and the unidentified ninhydrin positive compounds, did not show any definite distribution pattern corresponding to those of the two families studied. This probably implies that the free 'non-protein' amino acid pattern is not a suitable chemotaxon for the families Lythraceae and Melastomaceae. A point of interest in this study was the presence of an unusually high concentration of γ -aminobutyric acid (of the order of about 2.5% of the dry weight) in the seeds of Axinandra zeylanica Thw.