AMINO ACIDS LIBERATED INTO THE CULTURE MEDIUM BY PEA SEEDLING ROOTS

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INTRODUCTION

Rovira has shown that sterile culture medium in which pea seedlings have been grown contains amino acids. The present paper gives the results of a similar study using sterile pea seedlings; the amino acids have been identified and their amounts accurately determined using ion-exchange chromatography rather than the semi-quantitative paper-chromatographic methods used by Rovira. The free amino acids of the intact roots have also been determined.

EXPERIMENTAL

Seeds of *Pisum sativum* var. Alaska were obtained from a local seedsman.

Sterilisation and culture conditions

Seeds were placed in screw-capped bottles containing calcium hypochlorite solution (3% available chlorine) and shaken for 45 min. on a wrist action shaker. They were then half submerged in semi-solid glucose agar in screw-capped bottles and incubated at 25°C in the dark for five days. They were then examined and contaminated seedlings were discarded. The remainder were washed with three changes of sterile water and the seedlings divided into two groups. One group was placed in sterile boiling tubes containing acid washed quartz sand moistened with a nitrogen-free culture solution. The other group was placed in sterile boiling tubes whose walls were invaginated 2 inches from the base in four places so that the seedlings rested on the invaginations with the root submerged in the same culture solution as before. Both sets were plugged with cotton wool and partially embedded in sand so that on development the roots would be in darkness. The tubes were then incubated at 25°C with a light cycle of 12 hours light and 12 hours dark; the light intensity at tube level in the light cycle was 300 foot-candles and a
stream of air was blown over the tops of the tubes to minimise local heating. Eleven days after surface sterilisation a sample of culture solution was removed from each tube, plated on nutrient agar and incubated at 25°C for 3 days. The plates were then examined and any tubes shown in this way to contain contaminated peas were discarded.

**Preparation of materials**

The seedlings were harvested in batches of six and their root systems rinsed with water. In the case of seedlings grown in culture solution the rinsings were added to the pooled, filtered culture solution from six tubes. In the case of seedlings grown in quartz sand, the sand from six tubes was pooled and washed on a Buchner funnel with three successive volumes of water and the root rinsings added to this solution. Both solutions were then concentrated to 25 ml at 35°C on a rotary evaporater and an aliquot analyzed using a Technicon Amino Acid Autoanalyzer.

The roots of each batch of six seedlings were separated from the rest of the plant, lyophilised and weighed. Some batches of roots were ground with sand in a pestle and mortar with three successive volumes of 10 ml 0.01 M