Biochemical investigations on the coconut palm

I. A preliminary study on the amino acids in the coconut palm tissues

BY

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THE free and bound amino acids in plants have been well explored because of their importance in human and animal nutrition (Block and Bolling, 1951, Lakshminarayana Rao *et al.*, 1956). These investigations have also led to studies on their metabolic roles. Detailed studies using methods of chromatography, radioautography and spectrofluorimetry have revealed the presence of a number of amino acids and related compounds hitherto unnoticed in plants and have enhanced our knowledge of nitrogen metabolism and metabolic pathways. (Steward, *et al.*, 1955; Radhakrishnan *et al.*, 1955; Deshmukh and Sohonie, 1961). These aspects have been reviewed by Thomson *et al.*, (1959). Extensive studies on nitrogen metabolism have been carried out in a number of plants like barley (Richards and Burner, 1954), members of the family *liliaceae* (Fowden and Steward, 1957) and the banana (Steward, *et al.*, 1960) and proved to be very useful in bringing out the significance of amino acids and other nitrogenous constituents in growth and differentiation.

Our knowledge of the amino acids in the coconut palm tissues is very limited and is confined to those found in kernel and water (Baptist, 1956; Tulecka, *et al.*, 1961, Kimiyo Takeuchi, 1961; and Baptist, 1963). A systematic and detailed investigation on the amino acids in the various tissues under different ontogenic stages have therefore been initiated. The results of a preliminary screening are presented in this paper.

EXPERIMENTAL

The different tissues for the present investigations were collected from a coconut palm of the west coast tall variety. Chromatographic techniques were employed for the isolation and identification of amino acids.

EXTRACTION OF AMINO ACIDS

Coconut water

Equal volumes of nut water from nuts in different stages of development were concentrated to small volumes. These were passed through columns of cation exchange resin IR — 120, the sugars and other interfering substances washed off with glass distilled water and the amino acids adsorbed on the columns eluted with 2N ammonia solution. The eluates were evaporated to dryness and their residues taken up in equal volumes of water.

Kernel

Equal quantities of kernels in the developmental stages were extracted with 75 per cent ethanol, filtered and equal portions shaken up with three volumes of chloroform in separating funnels. The upper aqueous layers containing the amino acids were separated, evaporated to dryness and dissolved in equal volumes of water.

Leaf

Leaf samples collected from the different whorls of the crown were extracted as in the case of the kernel and further purified by IR-120.

Petiole, Stem and Root

Pressed juice of these were centrifuged and supernatent taken.

Protein hydrolysis

The residues of kernel and leaf after alcohol extraction were hydrolysed with 6N HCl for 1 hour in an autoclave at 15 lb. pressure. These were repeatedly evaporated with water to remove the acid and finally dried over potassium hydroxide in a vacuum desiccator. The residues were dissolved in known quantities of water, centrifuged and supernatent taken.

Chromatographic procedures

Aliquots of the different extracts were chromatographed on Whatman No. 1 filter paper using butanol: acetic acid: water (4:1:5) as solvent for circular paper chromatography. Whatman No. 3 filter paper bufferred with KCl - HCl buffer was used for two dimensional chromatograms, the solvents being phenol, pH 1 for the first run and butanol: acetic acid: water (4:1:1) for the second direction (Subramaniam and Lakshminarayana Rao, 1955). The amino acids were detected by spraying with a 0.2 per cent solution of ninhydrin in acetone and heating in an air oven at 60°C. The identifications were further done by spotting authentic samples side by side with tissue extracts, by specific tests and by observations under ultraviolet light.

RESULTS AND DISCUSSION

Circular and two dimensional chromatograms of the tissue extracts were examined for the different amino acids. Aspartic acid, serine, glycine, glutamic acid, threonine, alanine, tyrosine, valine, methionine, leucines, gamma-amino-butyric acid, proline, methonine sulphoxide, glutamine, asparagine, lysine, cystine, arginine, histidine and pipecolic acid were the prominent amino acids that could be detected in the different tissues of the coconut palm. The salient features noted in the distribution and concentration of amino acids were (1) the free amino acids in coconut water increased with maturity while that of the kernel and leaf decreased except in the case of gamma-aminobutyric acid which showed a regular increase with maturity of the kernel. There was no sharp change in concentration of bound amino acids. (2) While the protein fractions of kernel and leaf were rich in leucines, valine, methionine, tyrosine, glycine, serine, aspartic acid, alanine, lysine, histidine and arginine, the prominent free amino acids were alanine, gamma-aminobutyric acid, leucines, glutamic acid, threonine, glycine, serine and aspartic acid. (3) Asparagine could be detected in good amounts in tender leaves and roots. (4) Phenyl alanine was found only in the protein bound amino acids of kernel and leaves. (5) Pipecolic acid was found to be present in the soluble nitrogenous fractions of tender leaves and tendernut kernel. (6) The petiole, stem and roots were found to contain comparatively lower quantities of free amino acids. (7) Chromatograms of the extracts of stem and leaf when viewed under ultraviolet light gave a few fluorescent bands of light blue, pink, violet and green colour. The fluorescence got intensified on exposure to ammonia indicating the polyphenolic nature of the substances.

Chromatograms in figures I to IV illustrate some of these observations.

The present findings on the amino acids in kernel and water are in general agreement with the previous reports of Baptist (1956) and Tulecka, *et al.*, (1961). Hydroxyproline and gamma-aminobutyric acid could not be detected among the free amino acids as has been reported by Kimiyo Takeuchi (1961). The general decrease in the free amino acid content of the kernel and leaf reveals that these amino acids are utilized in the biosynthetic processes of the developing fruit and leaf. An estimation of free amino acid nitrogen by the formol titration method in the developing nut has shown that the free amino nitrogen decreased from 980 mg/100 g. to 98 mg. with maturity in the kernel while that in the water increased from 4 mg/100 cc. to 16 mg. These are in agreement with the results of chromatographic studies. Fluctuations of amino acids in developing organs have been noticed in other plants also (Grzesiuk, 1960). The presence of larger quantities of asparagine in tender portions reveals that this amide is a metabolite in sites of active growth. The occurrence of pipecolic acid in the growing regions is also interesting due to the fact that it is reported to be connected with lysine metabolism. The conversion of lysine to pipecolic acid in developing fruits has been established. (Deshmukh and Sohonie, 1961). The metabolic role of gamma-aminobutyric acid in water and kernel which appears with the formation of kernel and subsequently increases with maturity, is worth investigating. Gamm-aminobutyric acid is now be present in all higher plants with glutamic acid as its metabolic precursor reported to (Dixon and Fowden, 1961).

SUMMARY

A preliminary screening of free and bound amino acids in the entogenic stages of the leaf, kernel and water as also in the root and stem has been carried out. Twenty amino acids were detected in the different tissue extracts. In addition a few more ninhydrin reacting substances which appeared in two dimensional chromatograms are to be identified. The concentration of bound amino acids were more or less constant while the free amino acids were in a dynamic state. Presence of asparagine and pipecolic acid in tender tissues and the intensity and fluctuation of gamma-aminobutyric acid in the developing nut are the other salient features noticed during the investigation. Further studies on these as well as the nature of the fluorscent substances in the leaf and stem are expected to throw more light on the metabolic activities in growth and development of the palm.

This descriptive evidence of the amino acids present in the coconut palm provides for more quantitative work which is to follow.

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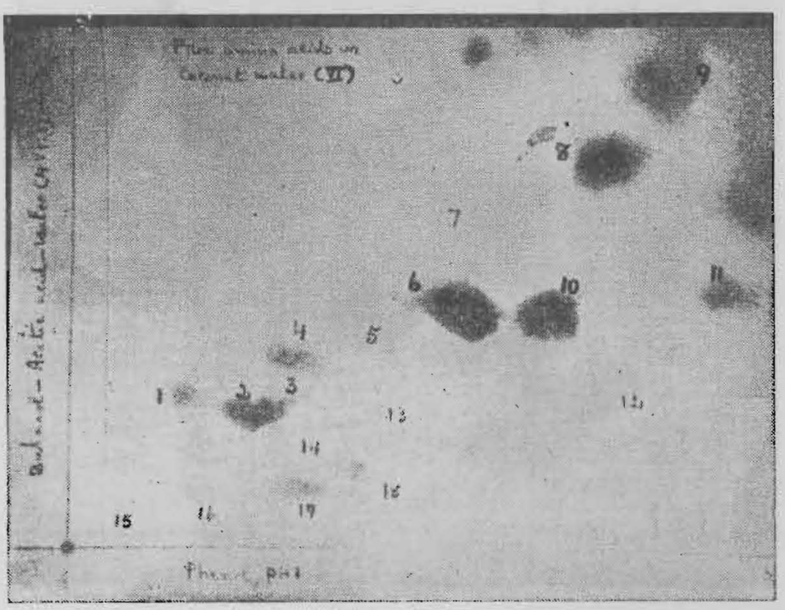

Figure I

1. Free amino acids in coconut water-Kernel just formed (Two dimensional Chromatogram).
2. Aspartic acid, 2. Serine, 3. Glycine, 4. Glutamic acid, 5. Threonine, 6. Alanine, 7. Tyrosine, 8. Valine, Methionine, 9. Leucines, 10. gamma-Aminobutyrine acid, 11. Proline, 12. Methonine sulphoxide, 13. Glutamine, 14. Asparagine, 15. Cystine, 16. Lysine, 17. Histidine, 18 Agrinine.

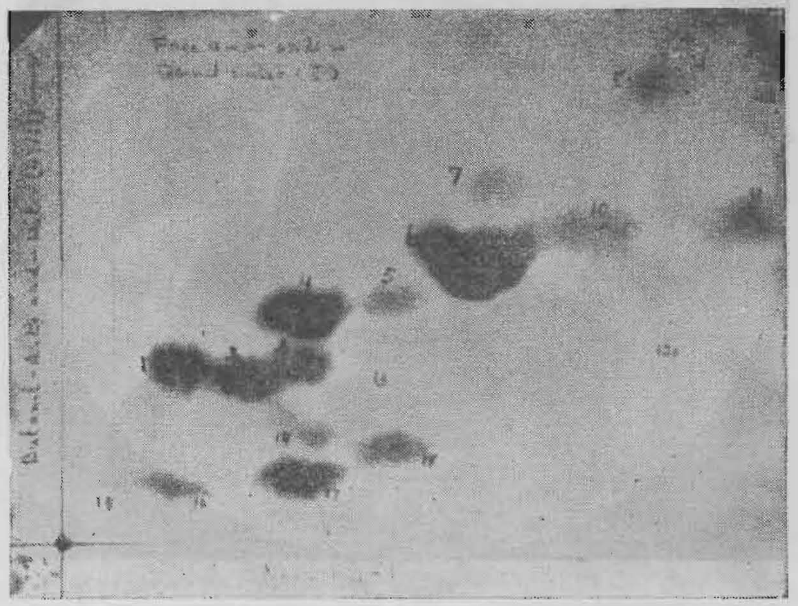

Figure II

Free amino acids in coconut water-fully mature nut-Spot Nos. 1 to 8 same as in Fig. I:

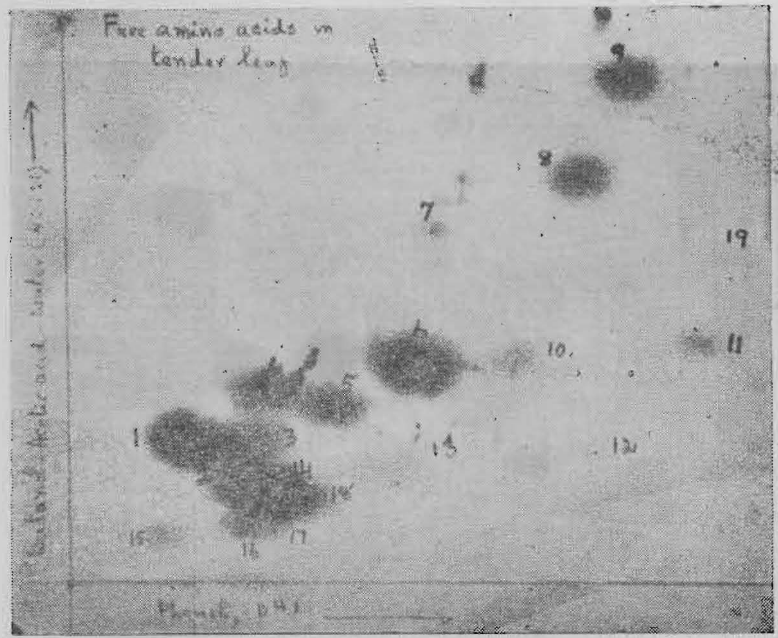

Figure III

Free amino acids in tender leaf. Spots Nos. 1 to 18,-same as in Fig. 1- Spot No. 19 pipecolic acid.

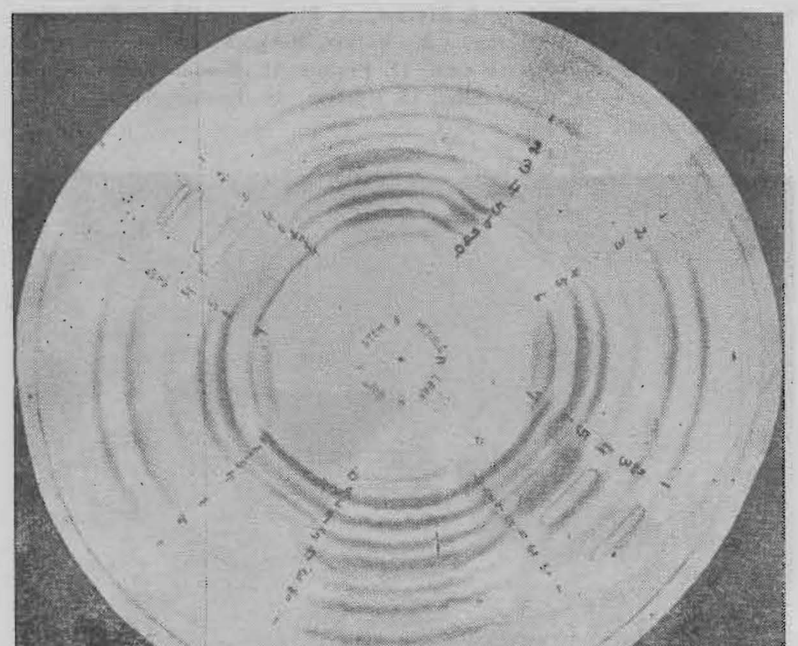

Figure IV

Free animo acids in Root, Stem, Petiole and leaf. The rounded spots show fluorescence under ultraviolet light.

A. authentic sample, I. Leucine, 2. Valine, 3. Trytophan, 4. Proline, 5. Threonine, 6. Glycine, Aspartic acid, 7. Lysine.

B. authentic sample: 1. Isoleucine, 2. Methionine, 3. Tyrosine, 4. Alanine 5. Glutamic acid, 6. Serine, 7. Arginine, 8. Histidine, 9. Cystine.