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Free Amino Acids in Three Genera of Sapindaceae†

by

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Introduction

In this paper we give details of some of the work we have been doing in the laboratories of the Botany Department of the University of Ceylon, Colombo. This work deals with the chromatographic identification of free amino acids present in three members of the family Sapindaceae. This family is included in the order Sapindales. We investigated three common genera belonging to this family. The technique used in this investigation was partition paper chromatography and column chromatography.

We selected :

(1) Nephelium lappaceum Linn, commonly known as Rambutan in Sinhala.

(2) Nephelium longana Camb, commonly known as Mora.

(3) Cardiospermum Halicacarbum commonly known as Penela Wel.

Our studies were confined to the amino acids present in the seeds of these plants.

The recent isolation of a free amino acid called α–Methylene Cyclopropyl glycine by Gray and Fowden of the University of London prompted our investigations. They have isolated this acid in Litchi chinesis. Hassall, Reyle and Feng also report a homologous Cyclopropyl derivative called β–Methylene Cyclopropyl Alanine also referred to as Hypoglycin A from Blighia Sapida. Both of these plants belong to the family Sapindaceae. We were interested in finding out whether any other genera belonging to this family possess a similar amino acid picture, at least homologous amino acids.

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Materials and Methods

A known quantity (usually 1 gram) of seeds were crushed and powdered. This was extracted with 75% Ethanol (*V*/*V*). Approximately 25 mls. of Ethanol to 1·0 gram of seed material were used. The extraction was done by shaking for a minimum period of sixteen hours at room temperature. The debris was centrifuged down and extracted with a further 25 mls. of 75% Ethnol. The combined supernatants were used directly as the Ethanolic extract.

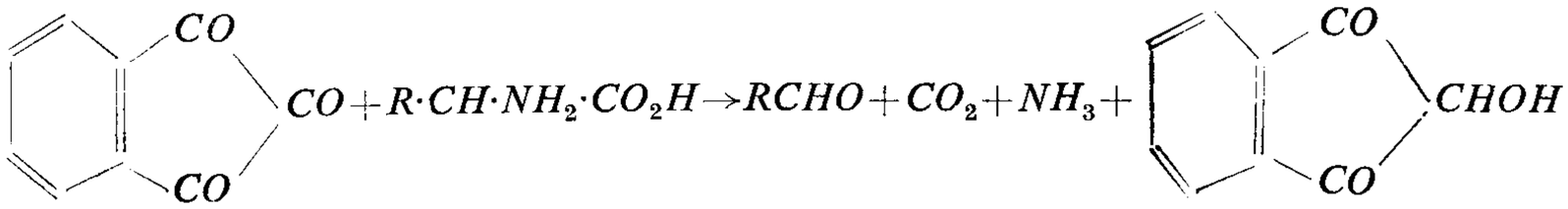
The extract was applied to a column of Zeo-Karb 225 (H^+ form activated in 2 *N HCl*). Non cationic substances were eluted with 75% Ethanol before the amino acids were displaced using 2 *N* Ethanolic Ammonia. The amino acid eluate was evaporated to dryness under a jet of air at room temperature. The residue was dissolved in a small volume of water before applying an aliquot to the paper chromatograms.

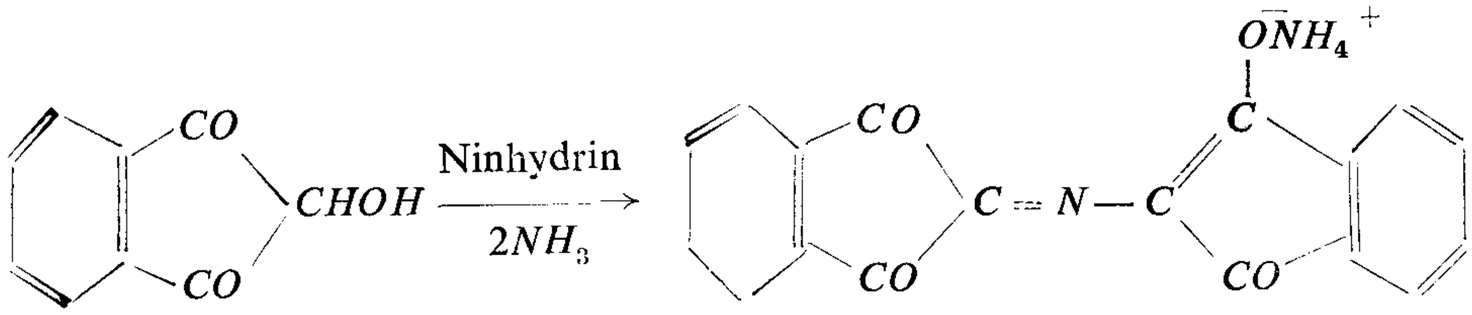
Grade I Whatmann chromatographic paper was used. Normally an aliquot equivalent to about 0·25 grams of seed was applied to a chromatogram. The amino acids were separated by two way chromatography at room temperature. In the first direction Phenol/water (3:1 *W*/*W*) in an atmosphere of Ammonia was employed. One phase mixture of N-Butanol/Acetic acid/water (90: 10: 29 *V*/*V*) was employed in the second direction.

0·1% Ninhydrin (*W*/*V*) in Ethanol was used for locating the amino acids while 2% solution of $NiSO_4$ in water (*W*/*V*) was used to spray the chromatograms so as to make spots permanent on the paper.

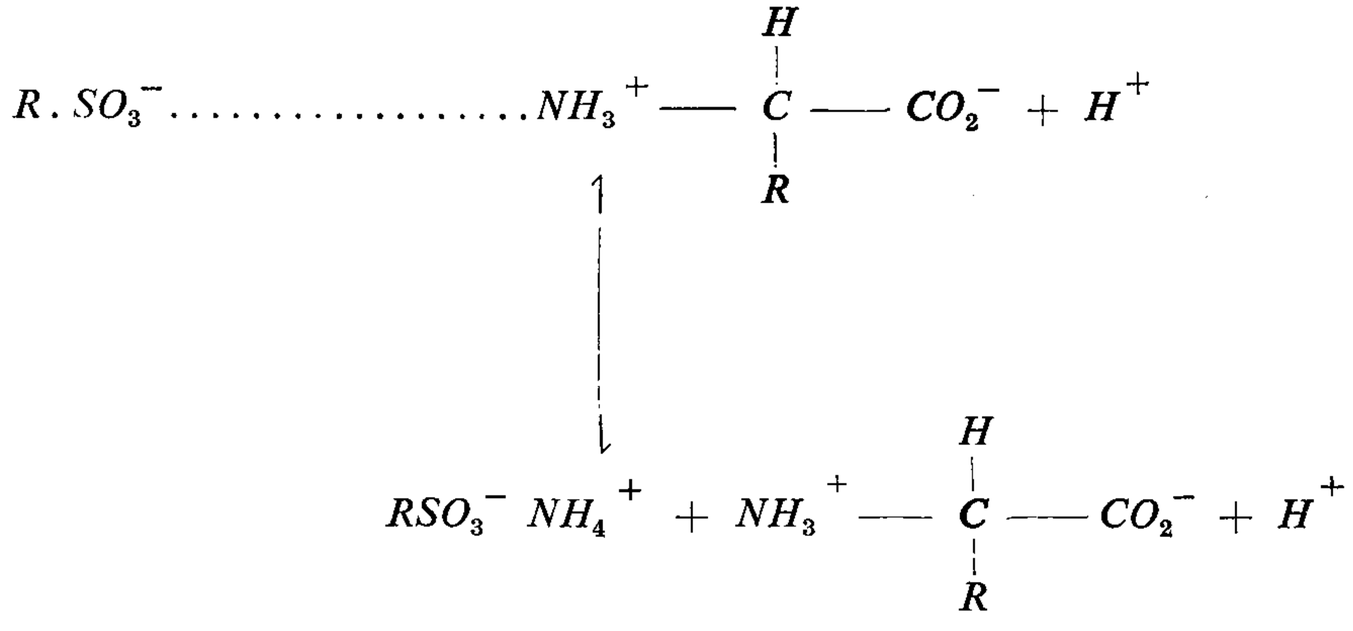
Ninhydrin reaction

Ninhydrin is Indane 1: 2: 3 trione hydrate.





Zeo-Karb prepared by nuclear sulphonation of cross-linked polystyrene. The sulphonic acid group are the only ionizable one present. Moreover, both the acid and the salt forms are ionized over the entire *pH* range, so that the resin is effective at any *pH*.



Results

Nephelium lappaceum (Rambutan)–Aril was not investigated, only the seed was used.

Amino acid picture

The picture does not show any unusual spots in any significant concentration.

3 faint spots

(1) Faint yellow spot–This occurs in all the three genera investigated.

(2) Dark blue spot–

(3) Bluish purple spot–pipecolic colour.

We have not attempted to identify these for two main reasons.

(i) Laborious time consuming procedure

(ii) Small concentrations

A very intense spot probably of γ-Amino butyric acid was found to occur. This could be identified by

(i) Its position

(ii) Sharp trailing edge

(iii) Diffuse leading edge

N. longana (Mora)

A new chromatographic spot was found. Unusual spots detected were,

(1) Brown spot – Probably is an Acetylinic amino acid.

(2) Another Acetylinic spot may be running along with Phenyl alanine.

(3) Yellow spot.

Cardiospermum Halicacarbum (Penela-wel)

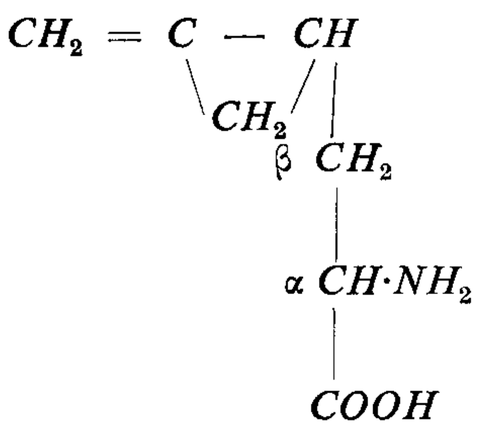
Unusual spots detected were,

(1) Orange brown spot which appeared little above Proline. This probably is another unsaturated free amino acid (Methylenic or Acetylinic)

(2) Yellow spot

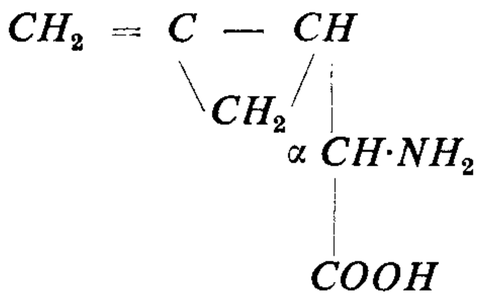
Discussion

Litchi chinensis and Blighia sapida have been analysed by other workers. In Blighia and Litchi they have found (Methylene cyclopropyl) L-alanine



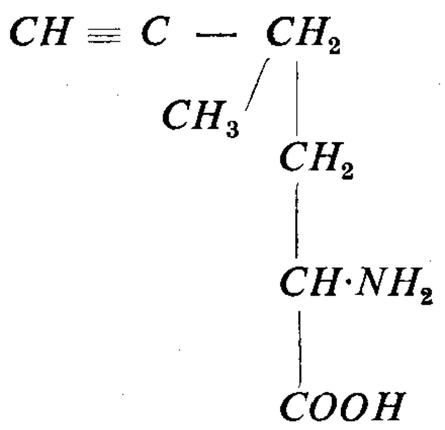
$[\alpha]_D = +9{\cdot}2°$ in water.

and (Methylene cyclopropyl) Glycine, respectively.

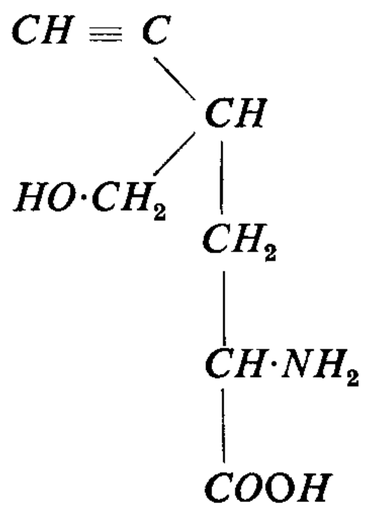


Both show hypoglycemic activity when injected to animals (Rats). But in our genera i.e., two Nepheliums and Cardiospermum, we did not detect any of the above two acids. It is definitely absent in N.lappaceum while it could be running with Phenylalanine in N.longana. We have communicated with one Chinese worker-(Miss Seng) working in London University, and she says that she has discovered three new Acetylinic amino acids from yet another member of Sapindaceae, called Euphoria longana.

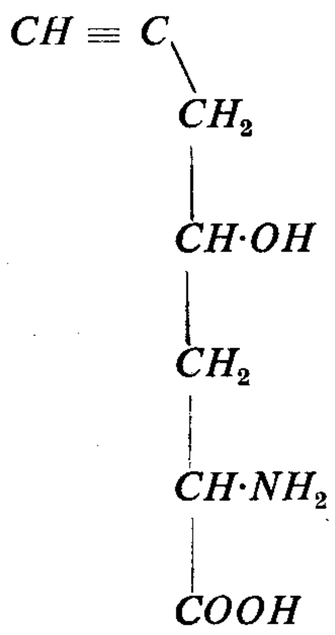
Three structures are as follows.



(2 Vinyl) propyl glycine
or
Pent-ynyl glycine
(1)



But. 1 yene 3 carbinol glycine
or
Pent-ynyl-ol glycine
(2)



n-Pent ynyl 5-ol glycine.

These are first examples of Acetylinic amino acids occuring in plants. The brown spot that we observed in N.longana probably is one of these acetylinic free amino acids [i.e., (2) above] judging by its chromatographic position. This brown spot turns purple after sometime or when strongly heated and this in reminiscent of the behaviour of (Methylene cyclopropyl) glycine. The other brown spot that we detected in Cardiospermum could be an amino acid that falls into the same group as the above five acids. This we infer by

(i) Its unusual colour

(ii) It occupies the same area in the chromatogram

(iii) Cardiospermum belonging to the same family is also likely to have a similar sort of compound.

Nephelium lappaceum seems to differ from the other 3 in that it does not have any of the above family of amino acids. On the other hand N.longana (Mora), Euphoria longana and probably Cardiospermum show some Acetylinic amino acids. This seems to suggest that they are more closely related to each other than any of them to N.lappaceum.

As it is only the amino acids that are of limited distribution e.g. Acetylenic amino acids here, that can be effectively used as systematic criterion we think these amino acids could be used with other morphological characters to study the relationships of genera in the family Sapindaceae. The amino acid patterns in various genera belonging to a family may also prove to be a useful taxonomic tool for instance to confirm the present morphological classification and also may throw light on doubtful situations existing in the present classification. This has been found to be so for Legumiosae. Lastly we would like to mention that apart from their chemotaxonomic significance these Acetylenic amino acids might show hypoglycemic activity just like Hypoglycin A and α (methylene cyclopropyl) glycine. If so they might be used to bring down the sugar level in Diabetics. Further research about these Acetylenic amino acids would be very useful along these lines.

Acknowledgement

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