Asian Journal of Chemistry Vol. 4, No. 1 (1992), 49–52

Free Amino Acids and Sugars of *Annona Squamosa* Linn.

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At least nine different amino acids and three free sugars have been isolated and identified from the leaves of *Annona squamosa* Linn (N. O.) *Annonaceae*), chromatographically and their relative occurrence has been evaluated colorimetrically.

INTRODUCTION

The leaves of *Annona squamosa* Linn (N.O. *Annonaceae*) are commonly used for the wormicidal action[1]. They are reported to contain an acrid principle fatal to insects[2] and used to kill lice[3]. Alcoholic extract of the leaves is reported to possess anti-cancer activity[4]. It is reportedly used for ulcers in the diabetic patients. Previous investigators reported the presence of alkaloids[5,6] and glycosides[7] in the plant. As amino acids are biogenetic precursors of several organic compounds, we attempted to isolate the free amino acids from the plant. Chemical investigations of the leaves of the young plant showed nine amino acids at least one of which possess significant role of biogenetic precursor for alkaloids. In this communication the isolation, identification and relative occurrence of these amino acids and some free sugars is reported.

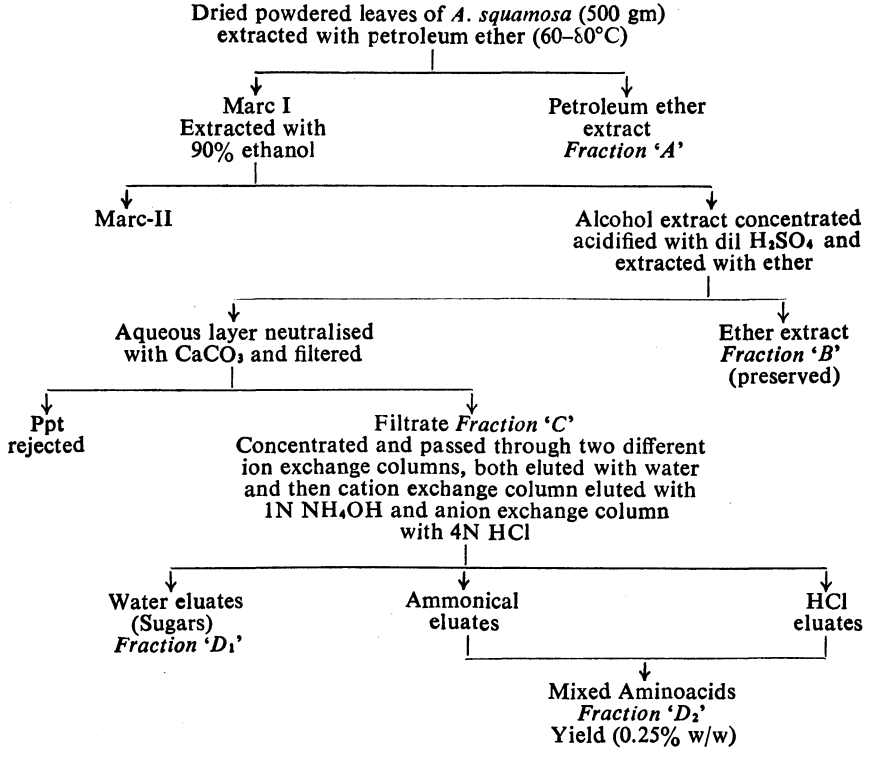
EXPERIMENTAL

Green leaves of the duly authenticated plant of *Annona squamosa* Linn. were collected during December/January from the surrounding areas of the nearby Junagadh district and were shade dried and powdered to 60 mesh. The bulk so obtained was used throughout the present investigations. The air-dried leaves of plant (500 gms) were milled and extracted in a soxhlet extractor with petroleum ether (60–80°C) and 90% ethanol successively for 48 hrs each. The alcoholic extract was concentrated and decolorised by refluxing with a little amount of activated charcoal. The slightly greenish filtrate was evaporated under reduced pressure to a gummy residue (15.2 gm).

The residue (1.5 gms) was demineralised by passing through a column of Amberlite IR 120(H)[8,9]. The column was washed with water till the eluate gave negative test with Molisch reagent for carbohydrates. The amino acids were then eluted with 4N HCl. The elution was continued till a drop of eluate failed to give bluish violet colouration on a filter paper moistened with ninhydrin reagent (0.2% in acetone). The total eluate was concentrated under reduced pressure and subjected to horizon-

tal paper chromatography[10] using n-BuOH—AcOH : H_2O (12 : 3 : 5) and PhOH : H_2O : NH_4OH (5 : 1.25 : 0.9) systems. Another portion of the alcoholic residue (1.5 gms) was also desalted by passing through Amberlite IRA 400 (OH) in the same manner as before. The amino acids were eluted with 1N NH_4OH. The eluate was concentrated under reduced pressure and paper chromatographed in solvent I. From both the fractions nine amino acids were identified.

SCHEME



All these amino acids from their mixture were isolated as individual ninhydrin complex by preparative —PC[11] on Whatmann (3 mm) paper using solvent I as developing solvent and ninhydrin as spraying reagent and heating the chromatogram at 60°C. The coloured bands were mapped out by pencil and R_f of each coloured band was compared with that of authentic samples. Then each band was cut out in small strips, and the strips were moistened with 75% ethanol (8 ml) in a petri dish for 30 min. The strips were then removed thoroughly washing with 75% ethanol. The solution in the petri dish was transferred in a colorimeter tube and ninhydrin reagent (1 ml) was added and warmed the mixture on a water bath for 15 min. The coloured solution was cooled to room tempera-

ture, made up to the volume to 10 ml with 75% ethanol. Optical density (O.D.) was measured colorimetrically at 540 nm[12]. The O.D. of proline was measured at 440 nm. The amount of each amino acid was obtained by interpolation of its O.D. in the standard curve of authentic sample.

The water eluate was concentrated (fraction D_2) and was spotted on a horizontal paper chromatogram. The spots of some standard simple sugars were also applied on the same chromatogram. Different chromatograms were developed using two different solvent systems *viz.*, n-BuOH : AcOH : H_2O (4 : 1 : 5) (horizontal) and *iso*-propanol : pyridine; water : AcOH (8 : 8 : 4 : 1) (ascending) and sprayed with different reagents like benzidine and aniline phthalate. Three brown spots were obtained with the test samples in each case.

RESULTS AND DISCUSSION

In all nine different amino acids were identified by their comparable R_f values with the authentic samples, as cystine, serine, argenine monohydrochloride, aspartic acid, 2-amino *n*-butyric acid, proline, methionine, β-phenyl alanine and *iso*-leucine.

The relative occurrence of individual amino acids (% w/w) in air dried leaves of the plant as obtained colorimetrically was cystine (0.20), serine (0.84), arginine monohydrochloride (0.54), aspartic acid (0.72), 2-amino-*n*-butyric acid (0.35), proline (0.50), methionine (0.40), β-phenyl alanine (0.50) and *iso*-leucine (0.24). Methionine is the source of both O- and N-methyl groups of many alkaloids and amino acids are also responsible for the glycosidic linkage with glucose and xylose as reported by Wagner *et al.*[5]

Three free sugars, *viz.*, glucose, fructose and xylose were also identified by their comparable R_f values with authentic samples. Their relative occurrence was of the order glucose, xylose, fructose based on the spot intensities.

ACKNOWLEDGEMENT

Authors are thankful to Dr. A. R. Parikh, Prof. & Head, Department of Chemistry, Saurashtra University, for his valuable guidance and permission to carry out this work.

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[*Received*: 23 November 1989; *Accepted*: 30 September 1990] AJC-227