Improved method for isolation of hypoglycins A and B from fruit of *Blighia sapida*

This report describes an isolation procedure for obtaining β-(methylenecyclopropyl) alanine, and its γ-glutamyl peptide (hypoglycins A and B) from ackee fruit or seeds (*Blighia sapida*) that is less laborious than previous methods (Hassall & Ryle, 1955; Ellington, Hassall & others, 1959; West, 1968) and gives leucine-free hypoglycin.

One kg of arilli from immature ackee fruit or of seeds from either immature or mature fruit were blended in batches with a total of 2 litres of 80% ethanol. After filtration through muslin and overnight settling the ethanolic extract was dried under vacuum, and the portion soluble in 0·1N HCl was applied to a column (90 × 4·5 cm) of Dowex 50 (×8) resin in the (H^+) form.

Several column volumes of 0·1N HCl and of water were passed through the column, after which elution with 1·0M pyridine until the ninhydrin test was negative removed neutral amino-acids, including hypoglycin A, as well as acidic amino-acids and hypoglycin B. The eluate was dried by evaporation under vacuum. The residue was taken up in 0·5N acetic acid and placed on a column (70 × 4½ cm) of Dowex 1 (×8) resin in the acetate form. Elution with 0·5N acetic acid rapidly removed a mixture of neutral amino-acids with hypoglycin A as the main component. This was recovered from the eluate after removing excess solvent by evaporation under vacuum. Crystallization, repeated twice from 50% ethanol, routinely yielded 0·8 to 1·2 g of nearly pure product per kg of starting material. Continued elution with 0·5N acetic acid removed a second zone of ninhydrin-positive material, well separated from the neutral amino-acids. This contained mainly hypoglycin B, present in seeds but not in arilli. This, in turn, was well separated from glutamic and aspartic acids, which appeared subsequently.

The identity of hypoglycin A was checked by nmr (Millington & Sheppard, 1968), and paper and thin-layer chromatography using a variety of solvent systems. However, for establishing the level of contamination by leucine and isoleucine, which exhibit similar solubility and chromatographic properties to those of hypoglycin A, it was necessary to perform chromatography of the derivatives formed with dimethylaminonaphthalene-5-sulphonyl chloride, using either filter paper (Abrahams & Kean, 1969) or polyamide layers (Woods & Wang, 1967). This technique was used to confirm that, by two or three recrystallizations of hypoglycin A derived from ackee arillus, contamination by these amino-acids could be reduced to less than 1%. In this respect, seeds were a less desirable starting material, in that the crude product contained relatively larger amounts of leucine and other amino-acids.

Hypoglycin B was identified from the products of hydrolysis (boiling with 8N acetic acid for 4 h), which were shown to be hypoglycin A and glutamic acid. Hypoglycin A with no detectable impurity was thus obtained in yields of up to 0·04% (based on the fresh weight of seeds used), by passage of the concentrated hydrolysate through a column of Dowex 1 (acetate form) as described above. Identification of the relevant compounds was readily achieved by thin-layer chromatography on silica gel (Eastman Chromatogram Sheets). The solvent system propanol–water (7:3) gave the R_F values: hypoglycin A, 0·73, hypoglycin B 0·51, glutamic acid 0·30.

The utility of the procedure lies in the selective elution, by dilute pyridine, of the neutral amino-acid and acidic peptide components in the extract, after adsorption on a strongly acidic cation-exchange resin. This fractionation could not be achieved with dilute ammonia. A solution of 1·0M pyridine is only weakly alkaline (pH 8·0) and this, together with the possibility of graded selectivity of the resin for different cations, including pyridinium, might explain the retention of the basic amino-acids.

Additionally, advantage was taken of the convenient fractionation of hypoglycins A (one amino and one carboxyl group free) and B (one amino and two carboxyl groups free), on a strongly basic anion-exchange resin by dilute acetic acid; this was based on the work of Hirs, Moore & Stein (1954).

This work was supported by a grant from the Wellcome Trust.

Department of Biochemistry,
University of the West Indies,
Kingston 7, Jamacia.

E. A. KEAN

January 18, 1974

REFERENCES

ABRAHAMS, D. E. & KEAN, E. A. (1969). *West Indian medl J.*, **18**, 147–151.
ELLINGTON, E. V., HASSALL, C. H., PLIMMER, J. R. & SEAFORTH, C. E. (1959). *J. chem. Soc.*, 80–85.
HASSALL, C. H. & RYLE, K. (1955). *Biochem. J.*, **60**, 334–339.
HIRS, C. W. H., MOORE, S. & STEIN, W. H. (1954). *J. Am. Chem. Soc.*, **76**, 6063–6065.
MILLINGTON, D. S. & SHEPPARD, R. C. (1968). *Phytochem.*, **7**, 1027–1029.
WEST, M. E. (1968). *J. Pharm. Pharmac.*, **20**, 974–975.
WOODS, K. R. & WANG, K-T. (1967). *Biochim. Biophys. Acta*, **133**, 369–370.

The binding of chlorpromazine to human serum albumin

The binding of chlorpromazine to human serum albumin (HSA) is a well established property (Curry, 1970). The kinetics of binding have been investigated by Jähnchen, Krieglstein & Kuschinsky (1969) using the techniques of equilibrium dialysis and gel filtration. They reported the following results: number of binding sites (n) = 30, affinity constant (k) = $2{\cdot}1 \times 10^4$ litre mol^{-1} (22°), pH 7·4. Nambu & Nagai (1972), however, using the same techniques reported: number of binding sites (n) = 2, affinity constant (k) = $7{\cdot}81 \times 10^3$ litre mol^{-1} (10°), pH 7·4. In order to resolve this apparent contradiction, we reinvestigated the kinetics of binding of chlorpromazine to HSA using the techniques of equilibrium dialysis and spectrofluorimetric quenching titration (Chignell, 1972). The results were evaluated using two different mathematical treatments, the Scatchard method (Scatchard, 1949) and the double log method (Thompson & Klotz, 1971).

Human serum albumin (HSA) was a lyophilized preparation for transfusion and a gift of the Manchester Blood Bank. Albumin solutions in Sorensen phosphate buffer (pH 7·4) were prepared immediately before use and assayed spectrophotometrically for albumin. Chlorpromazine hydrochloride (Largactil) was a gift of May and Baker Ltd.

Equilibrium dialysis. Dialysis was carried out using Visking Dialysis tubing (32/32) in a five compartment dialysis cell made to the specifications of Katz & Weissburger (1969). Drug concentrations in the range $2 - 10 \times 10^{-6}$ M and albumin concentrations of $6{\cdot}07 \times 10^{-6}$ or $12{\cdot}14 \times 10^{-6}$ M were placed on one side of the membrane and blank Sorensen phosphate buffer (pH 7·4) on the other. The cells were allowed to come to equilibrium by standing at 28° (± 1°) for 3 days and then the free drug concentration was determined spectrophotometrically using a Unicam SP 500 Series 2 spectrophotometer.

Spectrofluorimetric quenching titration. The titrations were made using a Baird-Atomic Fluorispec SF 100 E spectrofluorimeter. The excitation and emission wavelengths of HSA are 285 and 354 nm respectively and drug concentrations in the range