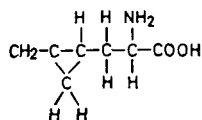


The isolation of L-leucine-free hypoglycin-A

SIR,—Hypoglycin-A, the toxic peptide from *Blighia sapida*, or ackee, a common article of Jamaican diet (Feng & Patrick, 1958), is known to have anti-sarcoma properties (Gaskin & Persaud, 1967). Hassall, Reyle & Feng (1954) and Feng & Patrick (1958) produced Hypoglycin-A (I) with L-leucine.



(I)

A new method has been found to prepare Hypoglycin-A free from L-leucine[†] a contaminant which antagonizes the anti-sarcoma properties (Gaskin & Persaud, 1967).

Material and method. 4.5 kg of mature seeds were ground and extracted with boiling water for 4 hr. The cooled filtrate was adjusted to pH 4 and proteinaceous material removed. The solution of mixed amino-acids was fractionated next on a Dowex 50W (x8) column (mesh 20–50, 60 cm × 5 cm). Elution was with N ammonia solution and the eluate was condensed to a small volume at 70° and then chromatographed on basic alumina Grade III (Merck) made up in acetone.

Development of this column was first with acetone and then acetone–water mixture, the latter solvent removing Hypoglycin-A from the column. The crystallized crude Hypoglycin-A was dissolved in warm water and further chromatographed on neutral alumina Grade III, from which 95% ethanol removed L-leucine, and a mixture of ammonia solution in ethanol removed Hypoglycin-A.

Hypoglycin-A was recrystallized from aqueous methanol and gave R_f 0.46 in a system of t-butanol–methanol–water (4:5:1). The yield was 0.03% w/w. The infrared spectrum in Nujol of the separated leucine was identical with that of authentic leucine, while that of the isolated Hypoglycin-A was identical with the synthetic material (Dr. S. R. Landor, private communications).

The nmr spectrum of Hypoglycin-A in D₂O at 25° is in agreement with the structure (I).

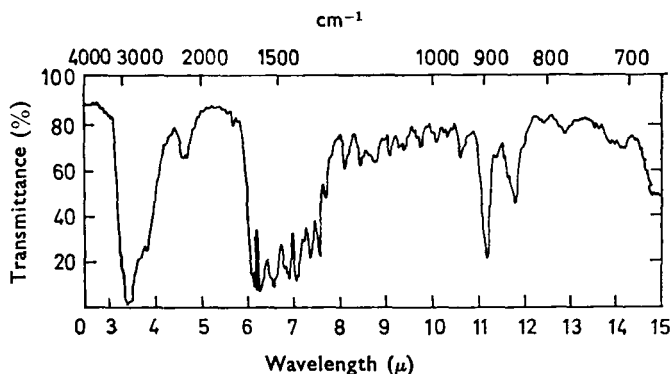


FIG. 1. Infrared spectrum of hypoglycin-A in Nujol.

Hypoglycin-A is one of the few natural products with this methylene cyclopropane ring system. The adsorption of both L-leucine and Hypoglycin-A on basic alumina took into account their respective solubilities in water, alcohol, acetone and pyridine. Both Hypoglycin-A and L-leucine are considered as neutral amino-acids, and should carry a negative charge in 90% ethanol and be retained by acid alumina (Wieland, 1942).

However their behaviour on acid alumina indicated that Hypoglycin-A and L-leucine had very different physicochemical properties, and selective elution from neutral alumina of Hypoglycin-A with ammonia solution-ethanol was based on the amino-acids classification of Schramm & Primosigh (1944).

The yield from this method was 0.03% w/w as compared to 0.008 to 0.01% w/w in earlier methods.

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