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SHORT COMMUNICATION

THE ISOLATION OF HYPOGLYCIN A AND RELATED COMPOUNDS FROM *BILLIA HIPPOCASTANUM*

J. N. ELOFF* and L. FOWDEN

Department of Botany and Microbiology, University College, London, W.C.1

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Abstract—Hypoglycin A has been isolated from seed of *Billia hippocastanum* and characterized by comparison with an authentic sample. Hypoglycin B (the γ-glutamyl peptide of hypoglycin A) and α-(methylenecyclopropyl)glycine were isolated in small amount and their identities confirmed by examination of the products of hydrolysis and catalytic hydrogenation respectively.

HYPOGLYCIN A [I, β-(methylenecyclopropyl)alanine] was isolated from unripe fruits of *Blighia sapida* (trivial name, ackee; family Sapindaceae) and shown to form the principal toxic component of these fruits, which can cause hypoglycaemia when injested by animals or humans.[1,2] This toxic condition, often known as vomiting sickness, has had a high incidence in Jamaica, where the syndrome is also termed ackee-ackee. Hypoglycin B, the γ-glutamyl peptide of hypoglycin A, contributes to the toxicity of unripe fruit,[2] presumably being hydrolysed rather readily in the body. Both substances occur in high concentration in seed of *B. sapida*, which also contains another cyclopropyl amino acid, *trans*-α-(carboxycyclopropyl)glycine, and its related γ-glutamyl peptide.[3,4] Although other amino acids showing a similar form of branching within a C_7 skeleton have been characterized in species of other genera forming the families Sapindaceae and Hippocastanaceae,[5] *Blighia* has remained the only known source of hypoglycin A. Now, we have recognized this substance as the major component of the free amino acid pool in seed of *Billia hippocastanum*: it is present at a level of about 5 g/kg dry seed, and 230 mg pure material were isolated from 90 g seed. The identity of the isolated sample with authentic hypoglycin A, obtained from *Blighia sapida*, was established by chromatographic comparison, by i.r. and NMR spectroscopy, and by optical rotational measurements. A similar mixture of homoleucines was obtained after catalytic hydrogenation of the two samples of hypoglycin A.

Small amounts of crude hypoglycin B and α-(methylenecyclopropyl)glycine (II) were isolated from the same extract of *Billia* seed. Neither of these substances was crystallized, but their identities were established by paper chromatographic comparison with authentic materials [hypoglycin B from *Blighia*, and α-(methylenecyclopropyl)glycine from *Litchi chinensis*[6]] using three solvent systems. In addition, the isolated hypoglycin B was shown to yield glutamic acid and hypoglycin A after mild acid hydrolysis, while α-(methylenecyclopropyl)glycine was converted by hydrogenation into a mixture of leucine, norleucine and *allo*isoleucine.[6]

* Permanent address: Department of Botany, University of Orange Free State, Bloemfontein, S. Africa.

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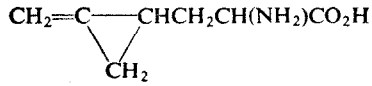
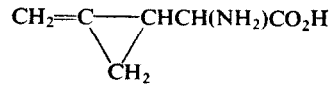
Billia hippocastanum is the only member of the families Sapindaceae and Hippocastanaceae known to produce both of the homologous compounds, α-(methylenecyclopropyl)glycine β-(methylenecyclopropyl)alanine (hypoglycin A). Their co-existence in this species suggests that hypoglycin A might be formed from α-(methylenecyclopropyl)glycine by a mechanism involving chain elongation (compare the conversion of valine into leucine); leucine and isoleucine each possess C skeletons that could act as the biogenetic precursors of α-(methylenecyclopropyl)glycine in a sequence of dehydrogenation reactions, one of which would effect the closure of the cyclopropyl ring. If these suggestions are correct, a careful re-examination of the amino acid complement of *Blighia sapida* should reveal the presence of α-(methylenecyclopropyl)glycine, at least as a trace component. Ultimately, it would seem more realistic to envisage that a critical biosynthetic study of hypoglycin A will be made on *Blighia*, which is a domesticated tree in Jamaica and Florida, rather than with *Billia*, whose seed was collected high on the slopes of a volcano in Costa Rica.



Hypoglycin A (I)



α-(Methylenecyclopropyl)glycine (II)

EXPERIMENTAL

Extraction and Characterization of Cyclopropyl Amino Acids

Dry *Billia* seed (90 g) was finely ground and extracted three times with 2-l. portions of 75% (v/v) ethanol. The combined extract was concentrated *in vacuo* at 40° and partially decolorized by treatment with absorbent charcoal. The cationic fraction was separated by absorption upon and elution from a Zeokarb 225 (×8) resin column. The combined amino acid residue obtained after evaporation of the NH_3-eluate was again decolorized and then applied as streaks across six sheets of Whatman 3MM filter paper. After developing the chromatograms in *t*-AmOH–HOAc–H_2O (20:1:20, v/v, upper phase), bands containing hypoglycin A, hypoglycin B and α-(methylenecyclopropyl)glycine were cut out and separately eluted with hot water. Hypoglycin A was crystallized after concentration, three batches of crystals (70, 110 and 50 mg) being obtained. The eluate containing hypoglycin B was re-chromatographed using 75% (w/w) phenol as solvent, while that containing α-(methylenecyclopropyl)glycine was purified by further chromatography in a *n*-BuOH–HOAc–H_2O (90:10:29, v/v). In this way samples of hypoglycin B and α-(methylenecyclopropyl)glycine, free of contaminating amino acids, were obtained.

The isolated sample of hypoglycin A had $[\alpha]_D^{20}$ + 11° (C = 2 in H_2O): literature value $[\alpha]_D^{23}$ + 9·1° (C = 1·1 in H_2O).[7] It gave an i.r. spectrum identical in every major respect with that obtained from an authentic sample of hypoglycin A isolated from *Blighia*, showing λ_{max} values of 887 and 1760 cm^{-1} (>C = CH_2), 1026 cm^{-1} (cyclopropane), and 1410 and 1590 cm^{-1} (CO_2^-) (compare literature).[7,8] Furthermore, the isolated and authentic samples of hypoglycin A (dissolved in NaOD–D_2O solution) gave matching NMR spectra, showing doublet, triplet and multiplet peaks in general agreement with those recorded previously[9] using a synthetic sample of hypoglycin A.

The isolate of hypoglycin B was inseparable from an authentic sample obtained from *Blighia* when run on paper chromatograms in each of the three solvent systems described above. After hydrolysis with N-formic acid at 100° for 2 hr,[2] glutamic acid and hypoglycin A were produced.

α-(Methylenecyclopropyl)glycine gives an initial brown-coloured spot when chromatograms are treated routinely with ninhydrin and so the chemical nature of this isolate was readily recognized. Further confirmation of the structure was obtained by demonstrating that the isolated material was converted into a mixture of leucine, norleucine and *allo*isoleucine (compare literature).[5]

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