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THE SYNTHESIS OF LOCUST ADIPOKINETIC HORMONE

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In 1976 Stone et al (1) reported the isolation and sequence determination of locust adipokinetic hormone (AKH). This blocked decapeptide (I), isolated from the corpora cardiaca of the insect, causes the release of diglycerides from the fat body into the haemolymph (2) and also stimulates the use of these lipids by the flight muscle (3).

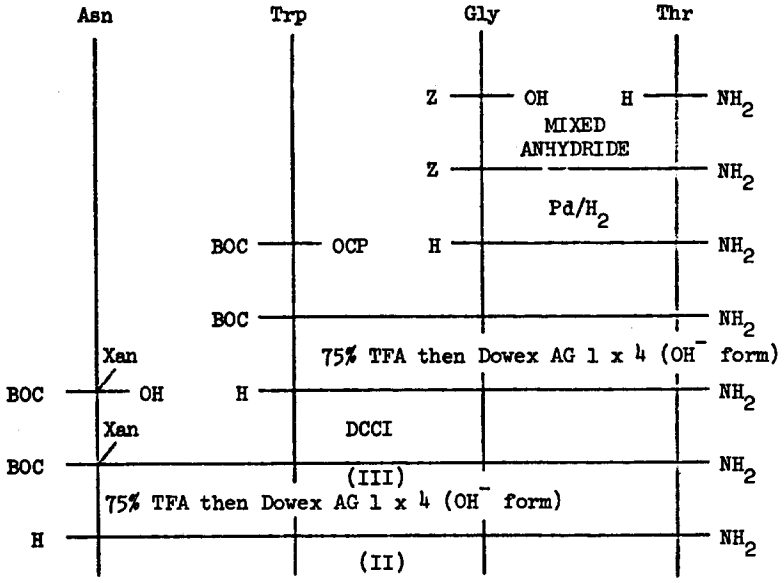
$$\square\text{Glu.Leu.Asn.Phe.Thr.Pro.Asn.Trp.Gly.Thr.NH}_2$$

(I)

This compound is the first peptide hormone from an insect neuroendocrine organ to be fully characterised, and opens up a new area in the chemistry of physiologically-active peptides. We now report a synthesis of this hormone carried out in order to confirm its structure and to make available adequate material for study of its mode of action. The synthetic strategy adopted involves a final coupling between the N-terminal hexapeptide, synthesised by the solid-phase method, and the C-terminal tetrapeptide amide (II) built up by a stepwise procedure from threonine amide (Scheme overleaf).

The solid-phase synthesis was carried out using Merrifield's resin (4), BOC-L-amino-acids and L-pyroglutamic acid, DCCI couplings, and 50% CH_2Cl_2/TFA deprotection, a fairly standard regime. A novel feature of the synthesis

was the use of the xanthydryl group for the protection of the side-chain amide of both asparagine residues during their coupling to prevent concomitant dehydration. Although this group has found some use in the protection of glutamine (5), it has not hitherto been applied to asparagine. During N-α-deprotection the xanthydryl group is also removed, but as β-cyanoalanine formation normally occurs only during coupling of the asparagine carboxyl group this is immaterial.



Scheme: BOC = t-butoxycarbonyl Z = benzyloxycarbonyl
Xan = xanthydryl OCP = 2,4,5-trichlorophenyl ester
DCCI = dicyclohexylcarbodiimide

Xanthydrol is known to react with tryptophan, indeed under acidic conditions it gives rise to a purple colour of use in detecting this amino-acid (6), but transxanthylation was not a problem on deprotection of the tetrapeptide III, although only a moderate yield (50%) of II was obtained. We were, however,

unable satisfactorily to deprotect BOC.Asn(Xan).Trp.NH_2, where intramolecular transfer may be facilitated by the proximity of the indole nucleus.

The N-terminal hexapeptide was cleaved from the resin with HBr-TFA and, after purification as its ammonium salt on superfine G25-Sephadex (0.05 M NH_4HCO_3), converted to the free acid (Dowex AG 50 x 4; H^+ form) and coupled to II in DMF using DCCI in the presence of 1-hydroxybenzotriazole (7), (94 μM scale). After evaporation of the solvent, trituration under 0.05 M NH_4HCO_3 and filtration, the filtrate was extracted with ether, degassed and loaded directly on to a Superfine G25-Sephadex column which was developed with 0.05 M NH_4HCO_3. The desired decapeptide eluted clear of the starting materials and coupling agents. Despite several attempts, we were unable to obtain a yield higher than 41 mg (38%; 87% based on tetrapeptide not recovered). This product, although homogeneous on tlc and gel filtration, had only ca. 70% of the activity of the natural hormone as regards fat-mobilising ability on injection into adult male Locusta. Hplc on a 30 cm μ-C_{18}-Bondapak column in methanol: water (41.5:58.5 v/v) (ca. 2000 theoretical plates) separated this product into two components (relative peak areas ca. 2:1). The faster running major component (44% based on tetrapeptide not recovered) had 99% activity on bioassay, and closely resembled AKH in its amino-acid analysis, hplc elution time, Rf on tlc (3 systems), mass spectrum, and in the electrophoretic mobility of fragments produced by thermolysin digestion. The structure of the closely related biologically inactive decapeptide contaminant is under investigation.

BOC.Asn(Xan).OH, m.p. 183-4 °C $[\alpha]_D^{27}$ + 2.1° (in DMF), was prepared in 38% yield by keeping BOC.Asn.OH and xanthydrol in glacial acetic acid for four days, evaporating, and crystallising twice from ethanol.

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