The Structure of Apamin†

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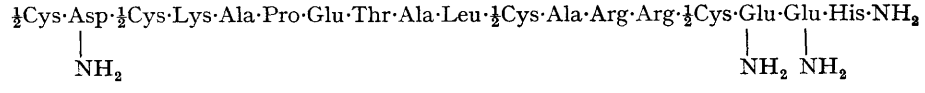
IT has been known for some years that bee venom contains an easily dialysable substance which has excitatory effects on the central nervous system.[1] Habermann and his colleagues[2] have shown that this substance is a peptide containing eighteen amino-acids ($\frac{1}{2}$Cys$_4$·Ala$_3$·Glu$_3$·Arg$_2$·Lys·His·Asp·Thr·Pro·Leu). They also showed that its pharmacological activity is destroyed by performic acid oxidation, and that no free sulphydryl groups are present. Consequently the molecule must contain two disulphide bridges. The German workers named the substance apamin.

Preliminary pharmacological tests (carried out by Dr. St. Shkenderov of the Research Institute for the Control of Medical and Biological Products, Sofia, Bulgaria) showed that injection of apamin (15—150 μg) into albino mice produces, initially, an increase in exploratory and motor activities. Later, movements become unco-ordinated and a general tremor appears. Clonic seizures, lasting up to one minute, affect the muscles, particularly those in the legs, and appear to be easily induced by a variety of external stimuli. The skin becomes dry and the respiratory rate increases.



In the course of an investigation of the components of bee venom, we have had occasion to isolate apamin and have determined its amino-acid sequence. Crude bee venom (obtained as a generous gift from "Rodopa", Sofia, Bulgaria) was subjected to forced dialysis: the dialysate was concentrated and applied to a column of Sephadex CM-25 at pH 4·6 (0·1M-ammonium formate). Apamin was eluted by using a concentration gradient (2·5M-ammonium formate). It was then concentrated and passed through a column of Sephadex G-25 at pH 4·6 (0·1M-ammonium formate). Final purification was achieved by using a column of Sephadex SE, also at pH 4·6 (0·1M-ammonium formate: gradient with 1·0M-ammonium formate). Amino-acid analysis of our product gave precisely the same results as those reported by the German workers.

Native apamin was subjected to tryptic hydrolysis and the resulting peptides were separated by ion-exchange chromatography. Three principal peptides were found. Amino-acid analysis and *N*-terminal analysis by the "dansyl" method[3] gave the following results:—

peptide 5

Ala·(Ala$_2$·Thr$_1$·Pro$_1$·Glu$_1$·Leu$_1$·$\frac{1}{2}$Cys$_1$)Arg.

peptide 4

$\frac{1}{2}$Cys·(Asp$_1$·$\frac{1}{2}$Cys$_1$)Lys.

peptide 10

$\frac{1}{2}$Cys·(Glu$_2$·His$_1$).

Free arginine was also found.

The amino-acid sequence was determined by the Edman degradation method in its three-cycle

† Application has been made for provisional Patent.

form.[4] Degradations were carried out on apamin which had been oxidised by performic acid and on apamin which had been reduced by mercaptoethanol and then carboxymethylated. Phenylthiohydantoin derivatives (P.T.H.) of the amino-acids were characterised by thin-layer chromatography on Eastman–Kodak silica gel sheets. Water-soluble P.T.H. derivatives were more easily identified from the reduced and carboxymethylated apamin, since the P.T.H. derivative of *S*-carboxymethylcysteine is soluble in organic solvents. The results were found to be consistent with the following sequence.

The positions of the disulphide bridges have not yet been established with certainty.

We thank the Medical Research Council for financial support (to A.F.B. and G.L.C.) and Dr. St. Shkenderov for carrying out pharmacological tests.

(*Received, March 15th*, 1967; *Com.* 249.)

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