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Procamine and Other Basic Peptides in the Venom of the Honeybee (*Apis mellifera*)

M. Larry Peck* and Rod O'Connor[1]

Honeybee venom is a complex mixture of substances, among them a number of basic peptides. Several of these demonstrate potent biological activity. Most recently the venom has been shown to contain a histamine-terminal peptide, procamine, which was the first such peptide isolated from a natural source. The synthetic preparation of procamine offers the opportunity of studying the biological properties of this previously unknown component of the venom.

The venom of the honeybee has been of interest to scientists for many years. A variety of medical uses for the venom have been suggested, the most widely known being in the treatment of certain arthritic conditions (Beck, 1935; Broadman, 1962), apparently through action of the venom in stimulating the pituitary–adrenal corticol system (Alfano *et al.*, 1973; Couch and Benton, 1972; Vick *et al.*, 1972). The effectiveness of bee venom in affording protection against radiation damage in mice (Ginsberg *et al.*, 1968; Shipman and Cole, 1967) has further stimulated investigation of the activities of major components of the venom.

Many persons evince severe reactions to bee sting, and death from a single sting, generally attributed to anaphylactic shock, is not uncommon (O'Connor *et al.*, 1964). The usual pattern in such cases is one of increasingly severe reactions to bee sting, even though these may be spaced over several years. Hyposensitization treatments have proved reasonably effective and injection of a pressor amine, such as epinephrine, within a few minutes of the onset of the anaphylactic reaction is recommended as emergency treatment (O'Connor *et al.*, 1964). The venom does contain toxic compounds, but the small amount injected in a single sting is of no real consequence in this respect. The allergic reaction is a serious problem and the wives of bee keepers are particularly susceptible, possibly by development of a hypersensitive condition from inhalation of the dust from clothes worn by their husbands while working with the bees.

The composition of bee venom is now reasonably well known (Table I) and a number of its components have

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Table I. Composition of Honeybee Venom

Components	% of dried venom[a] (approx)	Reference
Free amino acids (19)	1	Nelson and O'Connor (1968)
Histamine	1	Markovic and Rexova (1963)
Dopamine	?	Owen (1971)
Norepinephrine	?	Owen (1971)
Small peptides	15	
Seven di- and tripeptides	?	Rexova and Markovic (1963)
Histamine-peptides	2	(Figure 4)
Mellitins	50	Habermann (1972)
(three compounds)		Jentsch (1972)
Apamin	2	Habermann (1972)
MCD-peptide	2	Habermann (1972)
Minimine	?	Lowry *et al.* (1971)
Phospholipase A	12	Munjal and Elliott (1972)
Hyaluronidase	2	Barker *et al.* (1967)

[a] The natural venom is 88% water, by weight (O'Connor *et al.*, 1967), and contains a number of volatile compounds (Gunnison, 1966).

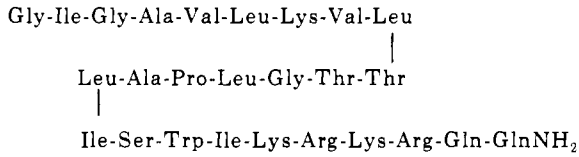


Figure 1. Mellitin I (Habermann, 1972).

been characterized and related to biological effects of the venom (Habermann, 1972). Of particular interest are two enzymes, phospholipase A and hyaluronidase, and four basic polypeptides, mellitin, apamine, MCD-peptide, and minimine.

The spreading of the venom through tissue is attributed to the enzymatic activity of hyaluronidase (Barker *et al.*, 1967) on the hyaluronic acid polymer, which serves as intercellular cement. Hyaluronidase, approximately 2% of the dried venom, has a molecular weight in excess of 20,000 and is one of the antigenic components of the venom. Unlike testicular hyaluronidase, the enzyme from bee venom has its optimum activity in the pH range from 4 to 5.

Bee venom phospholipase A (12% of dried venom weight) is a potent indirect hemolytic agent and produces a precipitous fall in arterial pressure and respiratory paralysis in test animals (Vick and Shipman, 1972). The enzyme is well characterized (Munjal and Elliot, 1972), having a molecular weight around 18,000, with 183 amino acid units. The N-terminal unit is isoleucine and a tentative sequence has been suggested (Habermann, 1972).

The principal peptides in bee venom are strongly basic (Neumann and Habermann, 1954). Mellitin, originally believed to be a single peptide, is now recognized as at least three closely related compounds (Jentsch, 1972) having strong direct hemolytic activity. Two of these have been completely characterized and produced synthetically (Lubke *et al.*, 1971). The mellitin peptides comprise about 50% of the dried venom and account for most of the toxicity. The structure of mellitin I is given in Figure 1. In addition to hemolytic activity, mellitins are associated with an increase in plasma cortisol levels (Vick and Shipman, 1972) and have pronounced surfactant properties.

Apamin (Figure 2) is the smallest known neurotoxic peptide (Habermann, 1972). Like mellitin, apamin produces an increased cortisol level in serum (Vick and Shipman, 1972). Although its potent and long-acting neurotoxic character has long been recognized, it has only recently been established as an antiarrhythmic agent (Vick *et al.*, 1972). Apamin is only about 2% of dried bee venom.

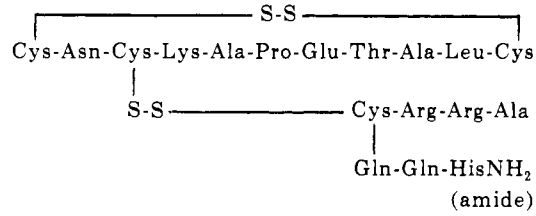
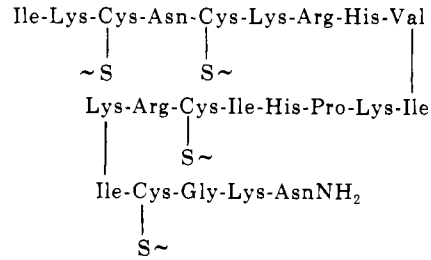


Figure 2. Apamin (Habermann, 1972).



Position of disulfide bridges not yet established.

Figure 3. MCD-peptide (Habermann, 1972).

Table II. First Synthetic Histamine-Peptides (Rocha e Silva, 1943)

N-Acetyldehydrophenylalanylhistamine
N-Acetyl-D,L-phenylalanylhistamine
N-Benzoyl-L-tyrosylhistamine
N-Benzyloxycarbonyl-L-tyrosylhistamine
N-Benzyloxycarbonyl-L-leucylhistamine

The venom contains a small amount (less than 1%) of free histamine. However, both phospholipase A and mellitin are capable of releasing histamine from the host organism. A third basic peptide (Figure 3), called the mast cell degranulating peptide (MCD-peptide), is several times as active as mellitin in releasing histamine from mast cells, so that it must be considered a prominent factor in the mastocytolysis resulting from bee sting (Habermann, 1972).

Recently a new peptide called minimine (Lowry *et al.*, 1971) has been found to possess a most interesting property. When injected into larvae of *Drosophila melanogaster*, minimine causes those larvae which develop into adult flies to be appreciably smaller, often one-fourth the size of normal adults. The progeny of these "miniature" flies are of normal size. Minimine has a molecular weight of about 6000 and, like other bee venom peptides, is strongly basic.

Two other components of known physiological activity have been reported, dopamine and norepinephrine (Owen, 1971). Together with the enzymes and peptides described, these contribute to the effects of honeybee sting.

PROCAMINE

In an effort to explain the inactivity of histamine present in cellular constituents and its release by proteolytic enzymes, Rocha e Silva (1943) suggested that histamine in the cell is bound by a peptide linkage and that such "bound" histamine should be physiologically inactive. The first histamine-terminal peptides were synthesized (Table II) and found to have little or no histamine-type activity. However, more recent evidence (Riley, 1959) indicates that histamine is bound electrostatically, rather than by peptide bond, to cellular components. Since the histamine-peptide model has not proved applicable and no naturally occurring histamine-terminal peptides had previously been reported, little is known of the physiological properties of such compounds.

Studies of small peptides in bee venom (Nelson and O'Connor, 1968) revealed the first natural source of histamine-terminal peptides. Two of these peptides have been

Ala-Gly-Pro-Gln—Histamine

Ala-Gly-Gln-Gly — Histamine

Procamine

Figure 4. Histamine-terminal peptides from honeybee venom (Nelson and O'Connor, 1968; Peck and O'Connor, 1973).

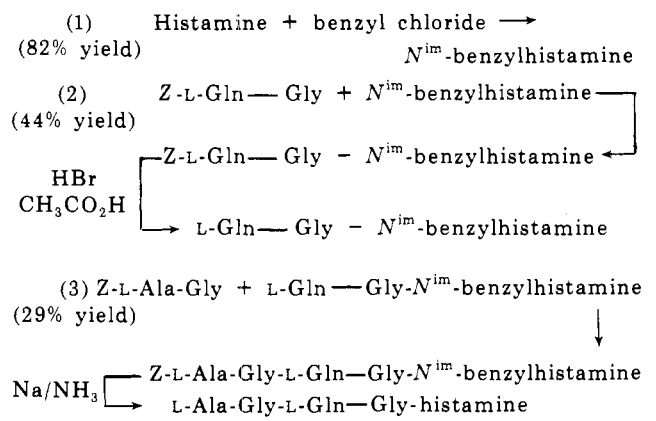


Figure 5. Synthesis of procamine (Z = benzyloxycarbonyl group).

characterized (Figure 4) and one, procamine, has now been produced synthetically so that physiological studies can be made on this new class of venom components.

STRUCTURE OF PROCAMINE

Initial isolation of procamine was from pure venom obtained by electrical excitation of individual bees (O'Connor *et al.,* 1963). For later studies requiring larger amounts, venom was obtained from John Toenyes of Power, Mont., and from Champlain Valley Apiaries of Middlebury, Vt., both using the method of Benton *et al.* (1963). Isolation utilized combinations of solvent extractions and preparative paper chromatography (Nelson and O'Connor, 1968).

Edman degradation (Edman, 1950) was used for the determination of amino acid sequence. In this procedure the peptide is treated with phenylisothiocyanate, forming the phenylthiocarbamyl peptide (at the original N-terminal unit). Cleavage of this product by anhydrous hydrogen chloride forms the phenylthiohydantoin (PTH) derivative of the original N-terminal unit and a peptide one amino acid smaller than the original. By repetition of this procedure, the peptide is degraded one amino acid at a time and the PTH amino acid produced after each cycle is isolated and identified.

The hydrolysis products of bee venom peptides are attacked by L-amino acid oxidase and unaffected by D-amino acid oxidase, indicating that amino acids in procamine were of L configuration.

SYNTHESIS OF PROCAMINE

Several synthetic routes were attempted, most resulting in poor yields due primarily to difficulties in incorporating histamine into the peptide. The best procedure devised, resulting in an overall yield of 10%, is outlined in Figure 5.

To avoid difficulties associated with the reactive imidazole ring, the imidazole nitrogen was protected by first forming the N^{im}-benzylhistamine by a method similar to that developed for histidine (du Vigneaud and Behrens, 1937). The blocked histamine was combined with *N*-benzyloxycarbonyl-L-glutaminylglycine (Melville, 1935), after which the benzyloxycarbonyl group was removed by treatment with hydrogen bromide in acetic acid. The resulting histamine-peptide was coupled with *N*-benzyloxycarbonyl-L-alanylglycine *via* the mixed anhydride method (Anderson *et al.,* 1967). Treatment of the product of this reaction with sodium in liquid ammonia resulted in the formation of procamine, which was purified by column chromatography.

The identity of the natural and synthetic peptides was established by amino acid analysis and both simultaneous chromatographic and cochromatographic comparisons.

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Another paper presented at the 165th National Meeting of the American Chemical Society in the Symposium on Toxic Proteins, Peptides, and Related Substances but not printed in this issue is: "Current Aspects of Research on Neurolathyrogens," by Charlotte Ressler, Kazunori Alie, and Tomohide Koga.