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Chemical Education

A CHIMIA Column

Topics for Teaching: Chemistry in Nature

The Sting's the Thing§

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Abstract: Bees defend themselves by stinging and injecting a venom into their victims; bee venom is a complex mixture of chemicals including the polypeptide melittin which is mainly responsible for triggering the pain of the sting.

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Bees belong to the order *Hymenoptera* and may be social or solitary insects. Social bees (so-called because they live in well-organized social groups) include honey bees which are essential for the pollination of plants including agricultural crops. Around half of all crops worldwide are pollinated by European honey bees (*Apis mellifera*, Fig. 1). Colonies of wild bees together with those kept in man-made beehives contribute to plant pollination, and bee-keeping has been carried out by man for *ca.* 7,000 years. However, in the 21st century, a combination of pesticides, parasites (notably the mite *Varroa destructor*), pathogens and declining areas dedicated to flowering plants is creating a crisis for European honey bee populations.[1] This article is about the well-established defense mechanisms that bees have evolved to deal with biological threats.

Bees use their stings as a defense and inject venom into their victims. A *venom* is defined as a toxic substance produced by an animal and which is injected through a bite or sting. Each female bee (worker bees and the queen of the colony) possesses a 'stinger' (Fig. 2) which is a highly adapted ovipositor. The stinger and venom sac are located within a chamber in the last segment of a bee's abdomen. When a bee is threatened, muscle contractions triggered by nerves cause the stinger to project from the bee's body, enabling the bee to deliver a sting within one second. In experiments using pig's skin, the force with which a bee stinger punctures the skin was found to be 2–3 mN.[2] This is significantly greater than the force of *ca.* 18 μN required for a mosquito to puncture human skin.[2] The scanning electron microscopy (SEM) image in Fig. 2b illustrates the barbs along the edge of the stinger. Because the barbs are arranged in a spiral pattern, the stinger screws into the victim,[3] and the presence of the barbs prevents its retraction from mammalian flesh. Thus, as a bee flies away after stinging its victim, it leaves behind its sting, attached muscles and venom sac and usually dies some hours later. Amazingly, the separated sting continues to automatically deliver venom and *ca.* 140–150 μg (dry weight) of venom is injected during a single bee sting. Multiple stings may be lethal – a non-allergic human with a body weight around 60–70 kg has a 50% chance of death when stung by 1,000–1,500 bees.[4] Allergic responses include anaphylactic shock.

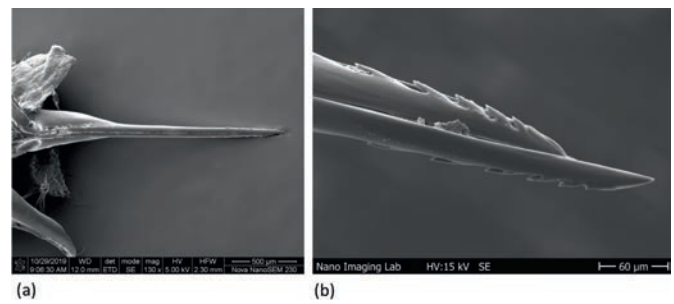
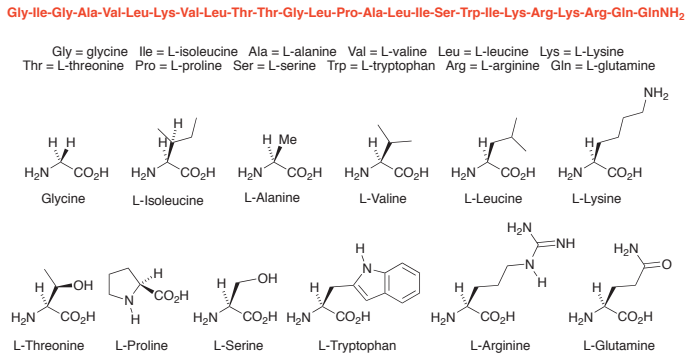


Fig. 2. Scanning electron microscopy (SEM) images of the stinger of a honey bee at different magnifications. The scale bar (bottom right) is (a) 500 μm and (b) 60 μm. The stinger is composed of *chitosan*, a linear polysaccharide. (Courtesy of the Nano Imaging Lab, SNI, University of Basel.)

Fig. 1. European honey bee (*Apis mellifera*). ©Edwin C. Constable 2019.

A single bee sting causes pain and swelling, but the chemistry of a bee sting is far from simple. The composition of bee venom was investigated at the end of the 19th century C.E., and it was wrongly concluded that formic acid was the main component.[5] In addition to water, bee venom contains proteins, polypeptides, amino acids, phospholipids, sugars, and volatile compounds including pheromones. *Melittin* comprises 40–60% of dry honey bee venom and is largely responsible for triggering a series of events which leads to the pain of a bee sting.[6] Melittin is a 26-amino acid polypeptide with the sequence shown in Scheme 1. The structures of L-alanine, L-leucine, L-isoleucine, L-lysine, L-serine and L-tyrosine, and the condensation of amino acids to form peptide links were detailed in an earlier *Chemical Education Column*.[7] Note that the IUPAC definition of a *polypeptide* is a peptide with 10 or more amino acid residues.[8]

The structure of melittin from a honey bee is shown in Fig. 3 and was determined by single crystal X-ray crystallography.[9] In the figure, two representations of a single polypeptide chain are shown. The chain has an α-helical coil (Fig. 3, bottom) supported by N–H...O=C hydrogen bonds (Fig. 3, top). In the crystal, the polypeptides associate to form tetramers (Fig. 4). In aqueous



Scheme. 1. The sequence of amino acid residues in melittin (top) and structures of the amino acids.

solution, melittin may be present as either a monomer or tetramer depending upon conditions such as concentration, pH and ionic strength. The amount of melittin in a single bee sting varies with the age of the bee. The sting of a 4-week old bee contains around 500 µg, but by 6 weeks of age, this has decreased to *ca*. 250 µg.[10] Melittin is able to bind to membranes forming pores which penetrate the membrane. This provides a passageway through which pain-inducing chemicals can pass.

In addition to melittin, bee venom contains the polypeptides adolapin and apamin as well as mast cell degranulating (MCD) polypeptide. Apamin and MCD-polypeptide constitute <3% of the dry-weight of bee venom and are 18- and 22-amino acid polypeptides, respectively. Both are neurotoxins[11] and MCD-polypeptide is a primary allergen in bee venom. The name MCD-polypeptide comes from the fact that it is responsible for breaking down mast cells in mammalian tissue leading to the release of histamine which causes inflammation.

The enzyme phospholipase A_2 makes up about 12–15% of the dry weight of bee venom. It is a polypeptide consisting of 134 amino acid residues and disulfide bridges that are structurally important in the folding of the polypeptide chain. The formation of disulfide bridges was described in an earlier *Chemical Education Column*.[12] Phospholipase A_2 acts in synergy with melittin. When a bee stings, injection of melittin causes membrane damage. After this primary event, the enzymic action of phospholipase A_2 results in cleavage of the phospholipid bonds present in the fatty acid part of the bilipid membrane.

While we associate bee stings with pain, bee venom has also been applied for therapy since early times, for example in ancient Egypt and China. *Apitherapy* is the name given to therapy which uses products from honey bees. Much research effort is currently focused on the use of polypeptides and enzymes from bee venom in treatments for inflammations and diseases of the central nervous system including Parkinson's and Alzheimer's diseases. Melittin also has antimicrobial properties.[11,13] Therapeutic applications of bee venom include those for skin diseases.[14]

In summary, in this column we have looked at some of the components of bee venom, in particular the polypeptide melittin which triggers the chemical reactions which lead to the pain we feel when stung by a bee.

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§This column is one of a series designed to attract teachers to topics that link chemistry to Nature and stimulate students by seeing real-life applications of the subject.

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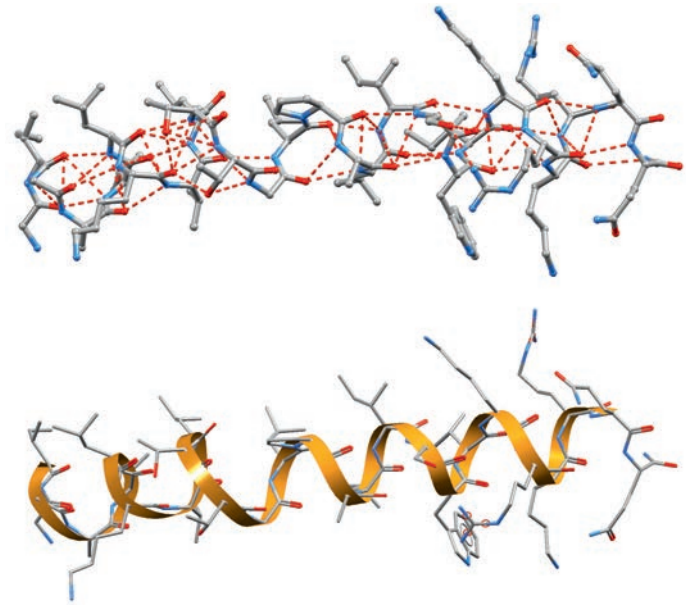

Fig. 3. The structure of the polypeptide melittin shown in both molecular and ribbon representations. Hydrogen atoms are omitted from the molecular representations, and the N–H...O=C hydrogen bonds are shown by the red hashed lines drawn between N and O atoms in the top figure. (Data: Protein Data Base, PDB, code 2MLT[9]).

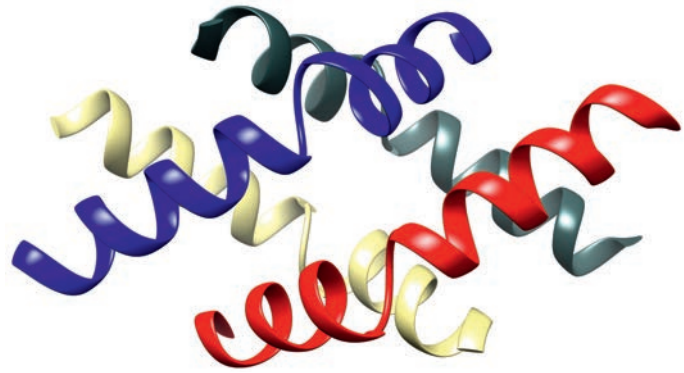

Fig. 4. The tetrameric structure of melittin shown in ribbon representation. (Data: Protein Data Base, PDB, code 2MLT[9]). Each α-helix corresponds to one polypeptide chain (see Fig. 3).

[1] G. E. Robinson, *Nature* **2019**, *571*, 34.
[2] Z.-L. Zhao, H.-P. Zhao, G.-J. Ma, C.-W. Wu, K. Yang, X.-Q. Feng, *Biol. Open* **2015**, *4*, 921.
[3] J. Wu, S. Yan, J. Zhao, Y. Ye, *PLoS One* **2014**, *9*, e103823.
[4] M. B. Pucca, F. A. Cerni, I. S. Oliveira, T. P. Jenkins, L. Argemi, C. V. Sørensen, S. Ahmadi, J. E. Barbosa, A. H. Laustsen, *Front. Immunol.* **2019**, *10*, 2090.
[5] R. O'Connor, L. Peck, *J. Chem. Educ.* **1980**, *57*, 206.
[6] J. Chen, S.-M. Guan, W. Sun, H. Fu, *Neurosci. Bull.* **2016**, *32*, 265.
[7] C. E. Housecroft, *Chimia* **2018**, *72*, 819.
[8] *https://doi.org/10.1351/goldbook*
[9] T. C. Terwilliger, D. Eisenberg, *J. Biol. Chem.* **1982**, *257*, 6010.
[10] H. Bachmayer, G. Kreil, G. Suchanek, *J. Insect Physiol.* **1972**, *18*, 1515.
[11] R. Wehbe, J. Frangieh, M. Rima, D. El Obeid, J.-M. Sabatier, Z. Fajloun, *Molecules* **2019**, *24,* 2997.
[12] C. E. Housecroft, *Chimia* **2018**, *72*, 428.
[13] M. Moreno, E. Giralt, *Toxins* **2015**, *7*, 1126.
[14] H. Kim, S.-Y. Park, G. Lee, *Toxins* **2019**, *11*, 374.