The Bee Venom Book

Eros, stung by a bee,
Ran away and cried for plea:
Venus, mother, I cry ,
Please help me or I'll die
What a terrible disgrace –
A dragon bit me on my face
Venus comforting her son
Speaking with a mocking fun -
The little bee's tiny sting
Is for you an earnest thing
But more painful and real hard
Are your stings in human's heart

Anacreontean songs, 6 BC

Venus, Eros and the bees

By A. Dürer, 1514

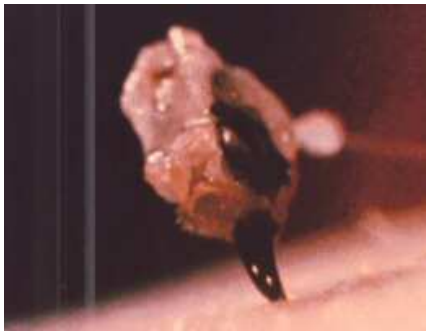
I would appreciate your feedback at http://www.bee-hexagon.net/contact/

Stefan Bogdanov, Muehlethurnen, Switzerland

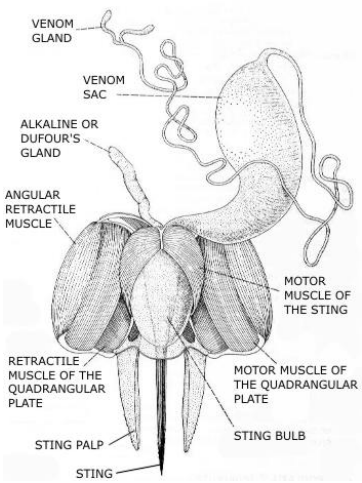
Bee Venom:

Production
Composition
Quality

Stefan Bogdanov



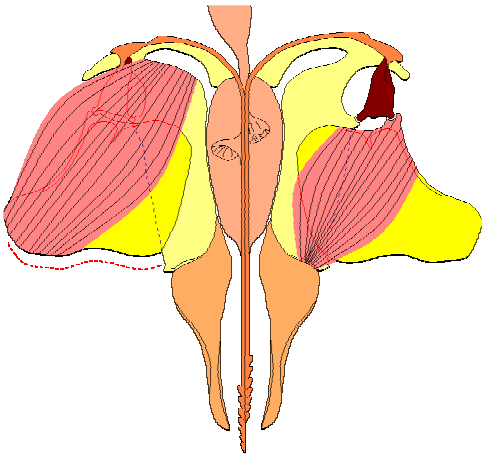
BEE VENOM PRODUCTION



Bees produce their venom in their venom glands, schematically described in the figure left. The BV is secreted in a branched acid gland (above) and in the alkaline Dufour's gland (below), in the whole BV both secretions are mixed.

New born bees do not sting. Venom synthesis begins after two or three days, while the maximal production rate is reached when bees are two to three weeks old. Older worker bees produce less venom. One sting contains about 100 μg of dry BV.

Drones do not have stings, while bee queens have BV, its maximum quantity being that of newly emerged queens, in order to facilitate their fight for survival against competing queens.



The sting consists of three parts: a stylus and two barbed slides (or lancets), one on either side of the stylus. The bee does not push the sting in but it is drawn in by the barbed slides. The slides move alternately up and down the stylus so when the barb of one slide has caught and retracts it pulls the stylus and the other barbed slide into the wound. When the other barb has caught it also retracts up the stylus pulling the sting further in. This process is repeated until the sting is fully in and even continues after the sting and its mechanism is detached from the bee's abdomen.

When stinging a mammal the sting is barbed so that it lodges in the victim's skin, tearing loose from the bee's abdomen and leading to its death in minutes. However, this only happens if the victim is a mammal or bird.

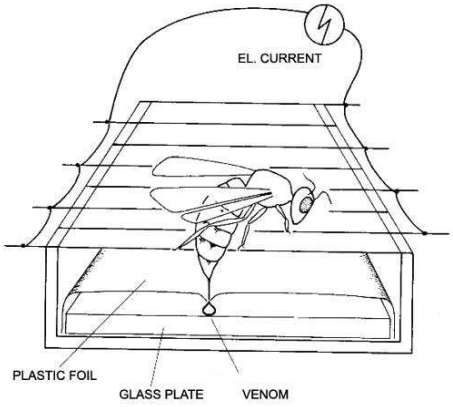
It has been speculated why bees lose their stings. The first theory says that the sting evolved early in evolution, before the appearance of mammals. Another theory says that both bees and mammals evolved at the same time. Losing the sting and dying during the fight with mammals is hypothesised to be more advantageous than the eventual loss of several worker bees because the bee can deliver a greater amount of BV into the victim. The alternative is the life loss of several worker bees[14]

All images were freely available in Internet, the sources could not identified

HARVEST OF BEE VENOM

Today BV is collected for commercial purposes by special collectors.

Collection of bee venom



Scheme of a BV collector, (after U. Mueller)

Most commercial venom collectors are composed of four parts:

- Battery or accumulator (24 to 30 V)

- Transformer from constant to alternating current, with impulse frequency of 50 to 1000 Hz and an impulse duration of 3 to 6 seconds.

- Collector frame consisting of an electric wire net and a glass plate, covered by a thin polyethylene membrane. The collector can be mounted in or out of the hive. The bees get in contact with the charged wire net and are stimulated to sting through the membrane and spray their venom on the glass plate. The glass plates are dried in a dark, well ventilated room.

Commercial BV collector

Beekeeper with a BV collector

An electric BV collecting technique was described for the first time in 1954 by Markovic und Mollnar[15]. Many different models have been developed[4, 6, 13, 17, 23]. The collectors have been used under different conditions:

- **Voltage**: from 24 to 30 V,
- **Impulse duration**: 2-3 seconds
- **Pauses**: 3 to 6 seconds; impulse frequency from 50 to 1000 Hz.

Bees are not harmed during the BV collection. Repeated 3 hours collection periods, carried out 3 to 4 times per month do not harm bees, resulting in a total harvest of 4 g dry BV. This collection resulted in a decrease of brood production and honey yield of about 10-15 %. If the collection was less frequent, e.g. 3-4 times per season, the bee performance was not influenced[13]. 10'000 bees are needed for the collection of 1 g dry BV[26].

BV is gathered commercially in Eastern Europe, Far East and North and South America.

BV from different honeybee species are slightly different but its overall activity is similar[12]

In warm and humid zones the BV can be more toxic than in cold temperate zones. Regarding the BV in different Apis types, it was found out that *A. melifera* and *A.dorsata* venom had similar toxicity, while *A. dorsata* venom contains much more alarm pheromones[26].

Different BV components can be isolated for special uses in medicine and biology.

PROPERTIES AND COMPOSITION

Properties of fresh bee venom, after [3, 5, 22]:

A bee venom drop

- The water content varies between 55 and 70 %.
- Yellowish opalescent liquid sometimes almost colourless,
  odour: honey-like; taste: aromatic, bitter, acidic and hot
- Soluble in water and diluted acids, insoluble in alcohol
- pH: 4.5-5.5
- Specific gravity: about 1.13
- Soluble in water, about 10 % are insoluble, water solutions are unstable, insoluble in ethanol,

dried BV: a gum like powder

The collected venom dries quickly in ordinary room temperature, turning into a yellow-brownish powder crystalline mass.

BV, relatively stable, destroyed by sun light and higher temperatures, stable at low temperature. easily destroyed by oxidizing substances: potassium permanganate, potassium sulphate; halogen elements-chlorine and bromine-destroy it very quickly; the effect of iodine is much slower. Alcohol possesses a strong and quick destructive effect on the venom. In contact with tincture of iodine, the alcohol is more destructive than the dissolved iodine, after[3, 20]

Composition

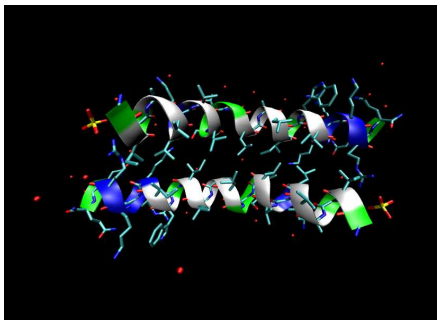
Bee venom is a complex mixture of proteins, peptides and low molecular components. Nowadays its components have been characterised. The main components are proteins and peptides. The different components are reviewed recently[1]. Here the most relevant components are given.

The composition of dry BV is given in the table below. The composition of fresh and dried BV differs mainly in regards to the volatile components; the overall biological activity is similar. The average dry weight per sting is 0.07 ±0.03 mg, the sting volume is between 10^{-2} - 10^{-3} ml[18]

Proteins (Enzymes)

The enzymes are proteins catalyzing specific reactions. There are 5 enzymes in BV.

Polypeptides



Polypeptides are smaller in molecular weight than enzymes, made of 2 or more amino acids. BV has numerous polypeptides (see table 1), the main one being melittin, which is also the main component of BV. Melittin has a MW of 2840 daltons but it can reach 12 500 daltons because it can be also in a tetrameric form[7-9]

The protein and the melittin electrophoretic patterns are typical of the honeybee species[12].

To the left: the structure of melittin (source: Wikipedia)

Low molecular compounds

BV contains smaller quantities of low molecular compounds are different in nature: amino acids, catecholamines, sugars and minerals. Sugars have been identified in some BV preparations, but if BV is collected with a collector preventing the contamination by pollen and nectar, it does not contain carbohydrates[22].

Table 1: **Composition of bee venom dry matter,** after [1, 2, 5, 18, 22, 26]

Substance Group	Component	% of dry weight
Proteins (Enzymes)	Phospholipase A2	10-12
	Phospholipase B	1
	Hyaluronidase	1-2
	Phosphatase	1
	α - Glucosidase	0.6
Peptides	Melittin	40-50
	Apamine	2-3
	MCD peptide	2-3
	Secapine	0.5-2
	Pamine	1-3
	Minimine	2
	Adolapine	0.5-1
	Procamine A, B	1-2
	Protease inhibitor	0.1-0.8
	Tertiapine, cardiopep, melittin F	1-2
Phospholipids		1-3
Biogenic amines	Histamine	0.5-2
	Dopamine	0.2-1
	Noradrenalin	0.1-0.5
Amino acids	Aminobutyric acid, α-amino acids	1
Sugars	Glucose, fructose	2-4
Volatiles (pheromones)	Complex ethers	4-8
Minerals	P, Ca, Mg	3-4

The composition of BV was last determined in 1966 by O'Conor et al[18]

QUALITY OF BEE VENOM

If it is not protected, oxidation will change the color from white to brownish-yellow. Changes caused by oxidation of certain components of the venom may decrease its healing effect. There are different kinds of venom such as: pure whole dried, whole dried and freeze-dried (lyophilized) BV. Pure whole dried BV is the purest venom. It is white in colour (often it is snow white), not contaminated with foreign materials and colourless when it is used in a solution. Optimal quality of BV can be achieved when it is harvested correctly. Contamination with bee faeces, dust, pollen, honey and other bee hive components should be avoided.

Freeze-dried BV is highly processed and purified venom. During the preparation its moisture content and any other contaminants are removed in order to purify and preserve it. Some of the active components may be removed also if an uncontrolled purification method is used. It is widely used in creams, liniments and ointments. In a tablet form, it can be used to prepare venom solution for electrophoresis or phonophoresis (ultrasound) applications. It is easy to sterilize by syringe filtration [24].

If BV is protected from moisture and light it can be stored for five years or more. It will not lose its toxicity, however its healing effects are reduced by storage. Freeze-drying is the most effective method of preserving BV.

Optimal quality of BV can be achieved when it is harvested correctly. Contamination with pollen, honey and other bee hive components should be avoided.

There is no international standard for BV quality. However, following quality criteria are followed by pharmaceutical firms:

Table 2: **Suggestion for a quality standard for dry bee venom**, according to the literature and after[16],[22]

Quality criteria	**Requirement**
Organoleptic properties:	typical
2 % BV solution:	Extinction at 420 nm smaller than 0.55
Water content:	less than 2 %
Water-insoluble substances	less than 0.8 %
Sugars	less than 6.5 %
Mellitin by HPLC	
Biological activity of hyaluronidase, phospholipase, melittin, protease-inhibitor	satisfactory
Radio-immuno tests	satisfactory
Toxicity	LD_{50} 3.7 ± 0.6 mg/kg*
* - LD_{50} – intravenous injection of a dose producing 50 % of mice survival	

Russian bee venom standard ФС 42-2683-89

Quality criteria	**Requirement**
Organoleptic properties:	typical
Water content:	less than 12 %
Water-insoluble substances	less than 10 %
% solids in the sol	less than 2
Hemolysis time	less than 480 seconds
Phospholipase activity units	less than 100
Hyaluronidase activity units	more than 70

Melittin and Apamin should also be determined in a future standard as they are the main biologically relevant components. Determination of melittin by HPLC in dry commercial BV in Iran showed variation between 8 and 51 % [11]or in a later study by the same authors it was measured in bee-hive collected BV in Iran, between 21.9 and 66.4[10].

LC-MS analysis of 5 Portuguese BV samples showed big variation of compostion in µg/ml: melittin: 36-86; phospholipase- 5-11; apamin: 0.9-1,9 (n=5) [25]

In a Chinese study with HPLC-DAD-MS/MS do determine melittin and apamin values of 46- 53 % and 2.2-3.7 % were found for both components (% of total dry weight) [27]

In Poland melittin and apiamin were determined by HPLC and following values were found in % of total dry weight: melittin: 61-70 %, apamin: 2.1 – 4.2[19]

PRODUCTION AND TRADE

BV is produced in many countries, mainly in Eastern Europe, South East Asia and the Americas. There are no official figures on the quantity of BV traded. Most of the BV is used for apitherapy and for desensitation in hospitals, but lately there is demand for the cosmetic industry.
Good commercial source of whole BV are www.beevenom.com and www.beevenomlab.com
Some companies produce and offer bee venom components such as melittin and apamin. A patent describing the production of alergene-free BV by removing PLA2 and hyalorunidase by ultrafiltration has been discribed[21]

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