site of action of apamin, a neurotoxic polypeptide of bee venom with an action on the CNS. *Biochemistry* **14**, 2521–2525.

Vladimirova, I. A. and Shuba, M. F. (1978). The effect of strychnine, hydrastin and apamin on synaptic transmission in smooth muscle cells. *Neirofiziologia* **10**, 295–299.

Vlasak, R. and Kreil, G. (1984). Nucleotide sequence of cloned cDNAs coding for preprosecapin, a major product of queen-bee venom glands. *Eur. J. Biochem.* **145**, 279–282.

Vogel, H., Järig, R., Hoffman, V. and Stümpfl, J. (1983). The orientation of melittin in lipid membranes. *Biochim. Biophys. Acta* **733**, 201–209.

Vogt, W., Patzor, P., Lege, L., Oldigs, H.-D. and Willie, G. (1970). Synergism between phospholipase A_2 and various peptides and SH-reagents in causing haemolysis. *Nauyn-Schmiedebergs Arch. Pharmakol.* **265**, 442–447.

Walde, P., Jäckle, H., Luisi, P. L., Dempsey, C. E. and Banks, B. E. C. (1981). Spectroscopic investigation of peptide 401 from bee venom. *Biopolymers* **20**, 371–385.

Wellhöner, H. H. (1969). Spinale Wirkungen von Apamin. *Naunyn Schmiederbergs Arch. Pharmakol.* **262**, 29–41.

Wemmer, D. and Kallenbach, N. R. (1982). Assignments and structure of apamin and related peptides in bee venom. *Biochemistry* **22**, 1901–1906.

Yunes, R., Goldhammer, A. R., Garner, W. K. and Cordes, E. H. (1977). Phospholipases: Melittin facilitation of bee venom phospholipase A_2 catalysed hydrolysis of unsonicated liposomes. *Arch. Biochem. Biophys.* **183**, 105–112.

Zurier, R. B., Mitnick, H., Bloomgarden, D. and Weissmann, G. (1973). Effects of bee venom on experimental arthritis. *Ann. Rheum. Dis.* **32**, 466–470.

8

Venoms of Bumble-bees and Carpenter-bees

TOM PIEK
Farmacologisch Laboratorium
Universiteit van Amsterdam
Amsterdam, The Netherlands

I. INTRODUCTION

Our present knowledge of venoms of bees other than the honey-bee is rather poor. When we look at Table II of Chapter 1, we see the Apoidea being subdivided into six families. Nothing is known about the venoms of the five first families of solitary bees. The last family, Apidae, can be subdivided into three subfamilies, two of which are interesting regarding knowledge of their venoms: the Apiniae, including the bumble-bees, the stingless bees and the honey-bees, and the Xylocopinae, or carpenter-bees. The venoms of the honey-bees are treated in Chapter 7; the venoms of the bumble-bees and carpenter-bees are treated in this chapter.

II. BUMBLE-BEE VENOMS

Comparing the lethality of hymenopterous venoms in mice, Schmidt *et al.* (1980; see also Chapter 9, Table XV) found the venom of the bumble-bee, *Bombus impatiens*, to have an LD_{50} of 7.2 $\mu g\ g^{-1}$ (95% confidence interval, 2.7–19). Therefore, the lethality of this bumble-bee venom may be comparable with those of honey-bees and many social wasps. Donnovan (1978) reported anaphylactic shock and strong cardiac stimulation caused by stings of *B. terrestris*.

Early reports on the venom components of bumble-bees were reported by Welsh and Batty (1963), who found very small amounts of serotonin in extracts of whole venom apparatuses of an unidentified *Bombus* species. It

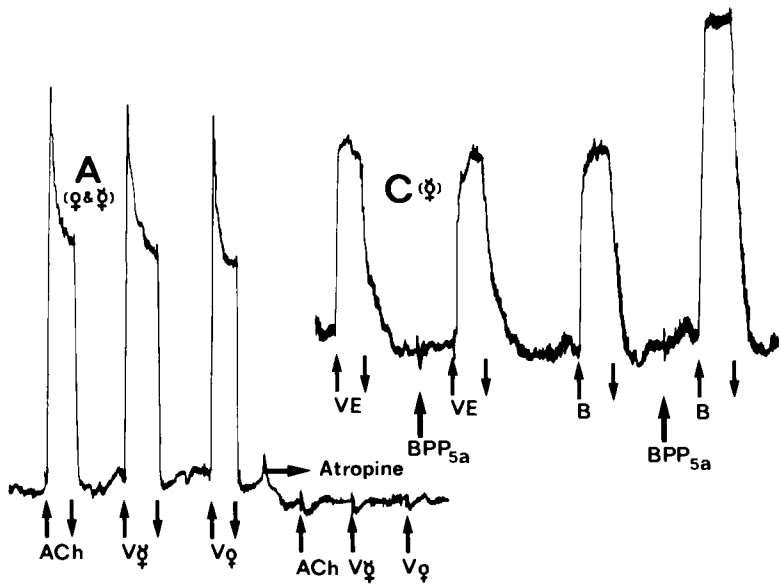
is not certain if this serotonin is a venom component. O'Connor *et al.* (1964) and Mello (1970) found proteins in the venoms of *B. huntii*, *B. occidentalis* and *B. atratus*, and Hoffman (1982) reported induction of allergy by stings of bumble-bees (see also Chapter 10, Section VI,A). Primary sensitization with *Bombus* venoms seems to be unusual. *Bombus* venoms are highly cross-reactive with honey-bee venom. At least four allergens from honey-bees can cross-react with similar allergens in *Bombus* (Hoffman, 1982)

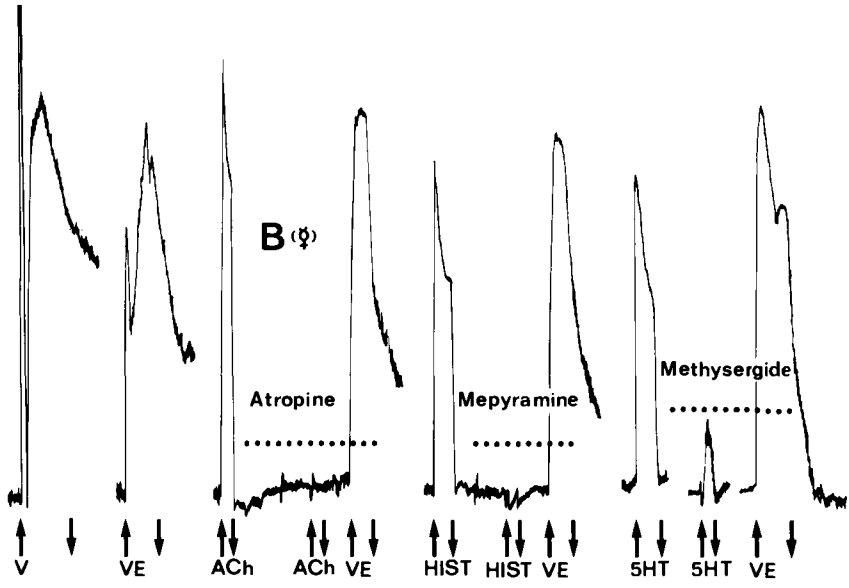
Nakajima (1979) has found putrescine in the venom of *Bombus ignitus*, and Piek *et al.* (1983) have found ~30 μg acetylcholine per venom reservoir (Fig. 1A) in the venom reservoir extracts as well as in dilutions of venom droplets collected from the tip of the sting of *B. terrestris*. The identity of the cholinergic factor with acetylcholine was confirmed using radioimmunoassay of acetylcholine.

The venoms of *Bombus terrestris* and *B. lapidarius* also contain a component that causes slow contraction of the guinea pig ileum and the rat diaphragm (Figs. 1B and 2) (Piek *et al.*, 1983). The ACh-like component of the mixed contraction of the ileum was greatly reduced after a 3-min treatment of the venom preparation with acetylcholinesterase (AChE). At a relatively high concentration the AChE-resistant component was not antagonized by atropine, mepyramine or methysergide (Fig. 1B). This suggests that the AChE-resistant component was not identical to a different cholinergic substance or to histamine or serotonin. The venom preparation [0.05 venom reservoir (v.r.) per milliliter] treated with cholinesterase caused a contraction of the ileum comparable in amplitude to that caused by 2 ng ml^{-1} of bradykinin, but in contrast to the bradykinin-induced contraction, the venom-induced contraction was not potentiated by BPP_{5a} (Fig. 1C), indicating that the slow contraction inducing substance is not bradykinin-like.

In the presence of 10 μg ml^{-1} of neostigmine the venom of *Bombus terrestris* causes a decrease in the twitch amplitude of rat diaphragm (Fig. 2A). In the absence of neostigmine such an effect was not observed (Fig.

Fig. 1 Effect of the venom of *Bombus terrestris* on isolated guinea pig ileum. (A) Low doses of venom (V) prepared from female (♀) and worker (☿) venom reservoirs (v.r.) cause contractions which are antagonized by atropine (3×10^{-5} v.r. ml^{-1} and 5×10^{-5} v.r. ml^{-1}, respectively) (100 ng ml^{-1}). ACh (1 ng ml^{-1}) causes a similar contraction. (B) A twenty times higher concentration also causes a slow contraction, which is more obvious when the venom is treated with cholinesterase (VE) (.01 v.r. ml^{-1}, 3 min treatment). The remaining slow contractions (VE at 0.1 v.r. ml^{-1}, 1 hr cholinesterase treatment) are not antagonized by atropine (100 ng ml^{-1}), mepyramine (20 ng ml^{-1}) or methysergide (10^{-6} *M*), which antagonize acetylcholine (ACh) (1 ng ml^{-1}), histamine (Hist) (10 ng ml^{-1}) and serotonin (5HT) (10 ng ml^{-1}) contractions, respectively. (C) The slow contraction by VE (.05 v.r. ml^{-1}) is not potentiated by the bradykinin potentiating factor (BPP_{5a}) (100 ng ml^{-1}), which potentiates the contraction by bradykinin (B) (2 ng ml^{-1}). Calibration: the ACh-induced contraction in A represents ~50% of the maximal contraction. From Piek *et al.* (1983).


A
(♀&☿)
C (☿)
VE
VE
B
B
BPP5a
BPP5a
Atropine
ACh
V☿
V♀
ACh
V☿
V♀


B (☿)
Atropine
Mepyramine
Methysergide
V
VE
ACh
ACh
VE
HIST
HIST
VE
5HT
5HT
VE

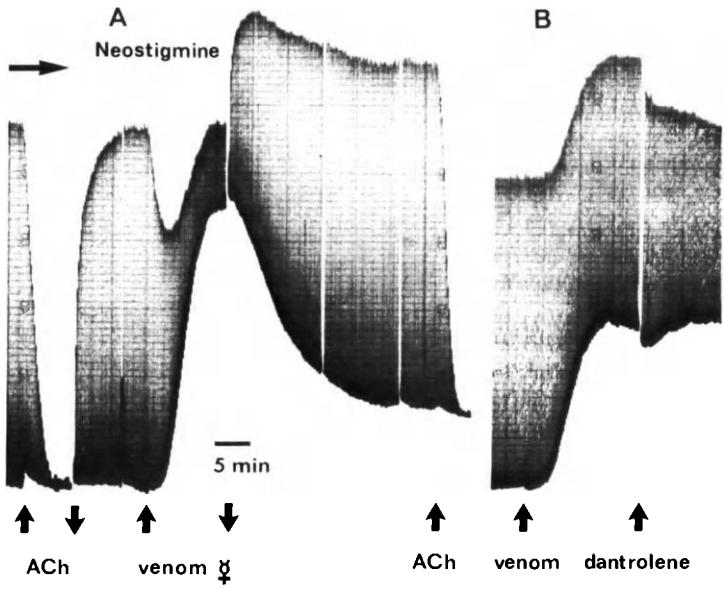


Fig. 2 Dual effect of the venom of workers (0.03 v.r. ml^{-1}) of *Bombus terrestris* on rat phrenic nerve-hemidiaphragm preparation at low temperature (19°C) and indirectly stimulated at 0.1 Hz. Horizontal arrow, time. (A) In the presence of 10 $\mu g\ ml^{-1}$ neostigmine a decrease in twitch tension, more or less comparable to that caused by acetylcholine (Ach) (1 $\mu g\ ml^{-1}$), is accompanied by a slow and tonic contraction. (B) Without neostigmine only the tonic contraction occurs. The venom-induced high tone is not decreased by dantrolene (15 mg $litre^{-1}$). From Piek *et al.* (1983).

2B). In addition to the cholinergic effect demonstrated in Fig. 2A, the venom also caused a slow tonic contraction of the diaphragm. In the absence of neostigmine, this slow contraction did not greatly affect twitch amplitude (Fig. 2B). Slow tonic contractions of rat diaphragm and guinea pig ileum occurred at comparable venom concentrations (Figs. 1 and 2). The slow contraction of the rat diaphragm is dose dependent and also occurs in the presence of *d*-tubocurarine. This may indicate that the substance causing slow contraction does not affect neuromuscular transmission, but some step following the transmission. The slow contraction is not affected by dantrolene (Fig. 2B). Therefore, the component might not affect the excitation–contraction coupling.

Dialysis for 3 hr at 4°C of 1 ml of venom solution in 0.9% NaCl against 10 litres of 0.9% NaCl did not decrease the total activity by more than 20%. The component that causes slow contractions is not dialysable, and the small decrease in activity can be explained by degradation, since at 0°C the activity

of the crude venom had fallen to 10% of the original value, probably due to enzymatic action. However, heating of the venom for 3 min at 100°C did not destroy its ability to induce slow contractions. The Ca^{2+} ion channel blocker verapamil antagonizes tonic contraction by the venom (Fig. 3); if it is added to the contracted diaphragm, a limited relaxation occurs. However, addition of verapamil prior to the venom treatment caused an enhanced contraction speed. It was concluded that this factor in the venom of *Bombus terrestris* is heat stable and nondialysable. This is an unusual combination of properties, since it indicates both low and high molecular weight.

Comparable contractions of rat diaphragm were found following treatment with the venoms of carpenter-bees, *Xylocopa violacea* (see next section), and the honey-bee, *Apis mellifera*, as well as with melittin from honey-bee venom. It has been found that melittin also does not pass through a dialysis membrane easily (see Chapter 7, Section III). This is explained by the fact that melittin is present in solution mainly as an aggregate (see Chapter 7, Section V,A,4).

Therefore it is probable that the above-described factor in the venom of *Bombus terrestris*, which is responsible for the slow contraction of visceral and skeletal muscles, is a melittinlike substance. Arguments in favour of this notion are (1) the similarity in contraction type produced by both *B. terrestris* venom and melittin, (2) the factor causing slow contractions, as well as melittin, does not pass easily through a dialysis membrane, which is unusual for substances which are heat stable and (3) using thin layer chromatography on cellulose with butanol–acetic acid–water (4:1:2) as an elution medium, the slow contraction-causing factor from *B. terrestris* venom could not be distinguished from melittin (H. W. Spanjer, personal communication).

Argiolas and Pisano (1985) have found five structurally related heptadecapeptides in the venom of the bumble-bee *Megabombus pennsylvanicus*. They have named them bombolitin I (Ile-Lys-Ile-Thr-Thr-Met-Leu-Ala-Lys-Leu-Gly-Lys-Val-Leu-Ala-His-Val-NH_2), bombolitin II (Ser-Lys-Ile-Thr-Asp-Ile-Leu-Ala-Lys-Leu-Gly-Lys-Val-Leu-Ala-His-Val-NH_2), bombolitin III (Ile-Lys-Ile-Met-Asp-Ile-Leu-Ala-Lys-Leu-Gly-Lys-Val-Leu-Ala-His-Val-NH_2), bombolitin IV (Ile-Asn-Ile-Lys-Asp-Ile-Leu-Ala-Lys-Leu-Val-Lys-Val-Leu-Gly-His-Val-NH_2) and bombolitin V (Ile-Asn-Val-Leu-Gly-Ile-Leu-Gly-Leu-Leu-Gly-Lys-Ala-Leu-Ser-His-Leu-NH_2). Bombolitins lyse erythrocytes and liposomes, release histamine from rat peritoneal mast cells and stimulate phospholipase A_2 from different sources. The threshold dose is 0.5–2.5 $\mu g\ ml^{-1}$ depending on the peptide and the bioassay. According to Argiolas and Pisano (1985), bombolitin V is as potent as melittin from honey-bee venom in lysing guinea pig erythrocytes ($ED_{50} = 0.7\ \mu g\ ml^{-1} = 4 \times 10^{-7}\ M$) and five times more potent than mastoparan in causing mast cell degranulation, making it one of the most potent degranulating peptides discovered so far ($ED_{50} = 2\ \mu g$

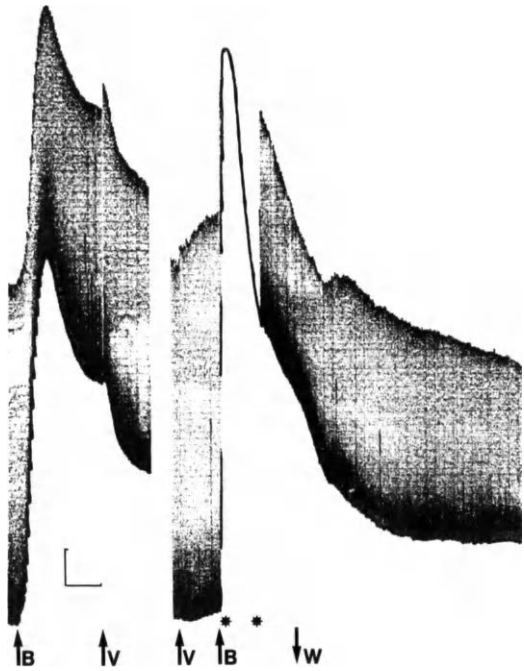


Fig. 3 Effect of verapamil on tonic contractions of isolated rat hemidiaphragm preparation, induced by heated (3 min, 100°C) and filtered venom preparation from *Bombus terrestris*. The diaphragm is stimulated indirectly (0.1 Hz), except between the asterisks. Left: the limited antagonistic effect of 10^{-4} *M* verapamil (V) added to the bath 10 min after the venom (B) (0.1 venom reservoir (v.r.) ml^{-1}). Right: administration of verapamil prior to the venom caused a potentiation of the contraction speed by the venom. W, Wash. Calibration: 5 min and 10^3 *N*.

$ml^{-1} = 1.2 \times 10^{-6}$ *M*). The bombolitins represent a unique structural class of peptides and have the same biological properties as melittin (from honeybees) (see Chapter 7), mastoparan (from wasps, hornets and yellow jackets) (see Chapter 6) and crabolin, a tridecapeptide isolated by Argiolas and Pisano (1984) from the European hornet *Vespa crabro* (see Chapter 6). The fact that these different peptides have the same biological properties may be caused by their amphiphilic nature, a property these peptides have in common.

It would be interesting to know whether all members of the Apinae produce melittin- or bombilitin-like peptides in their venoms, and how these peptides may differ from each other in structure.

III. CARPENTER-BEE VENOM

The venom of the solitary bee *Xylocopa violacea* was first described by Bert (1865), who observed that the sting of these bees could kill small birds within a few hours. In humans the sting of *X. violacea* (Hardouin, 1948) and that of *X. virginica* (Hermann and Mullen, 1974) seems to be extremely

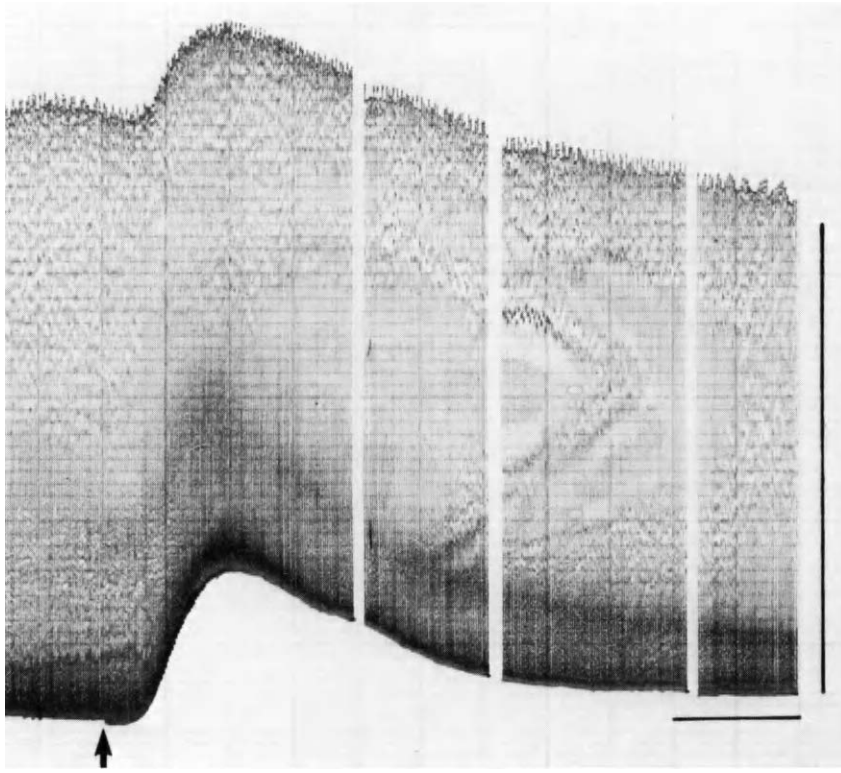


Fig. 4 Effect of the venom preparation of female *Xylocopa violacea* (0.3 venom reservoir (v.r.) ml^{-1} bath fluid) on twitch contractions (0.1 Hz) of phrenic nerve-hemidiaphragm preparation of the rat. The venom is added at the arrow; gaps in the record represent periods of wash. Calibrations: 5×10^{-2} *N* and 10 min, respectively.

painful. It causes local paralysis and oedema (for lethality in mice, see Chapter 9, Table XV). Nakajima (1979) demonstrated that the venom of *X. appendiculata* contains about 5 nmol histamine, 5 nmol putrescine and 2 nmol spermidine per venom reservoir. We have found in the venom of *Xylocopa violacea* a histamine-like activity comparable to ~ 300 ng of histamine per venom reservoir and in the much smaller species *X. canescens* ~ 40 ng per venom reservoir. Contraction of guinea pig ileum was fully antagonized with 10 ng ml^{-1} of mepyramine.

In rat phrenic nerve-himidiaphragm preparation the venom of *Xylocopa violacea* caused a slow tonic contraction, which could not be maintained completely and was also slowly reversible. This type of slow contraction (Fig. 4) is completely similar to that caused by the venom of *Bombus terrestris* and by melittin from *Apis mellifera*, indicating that here too a melittin-like substance may be present in the venom.

REFERENCES

Argiolas, A. and Pisano, J. J. (1984). Isolation and characterization of two new peptides, mastoparan C and crabrolin, from the venom of European hornet, *Vespa crabro. J. biol. Chem.* **259**, 10106–10111.

Argiolas, A. and Pisano, J. J. (1985). Bombolitins, a new class of mast cell degranulating peptides from the venom of the bumblebee *Megabombus pennsylvanicus. J. biol. Chem.* **260**, 1437–1444.

Bert, P. (1865). Contributions à l'étude des venins. II. Venin d'abeille xylocope. *Gazette Méd. Paris* **20**, 770–771.

Donnovan, B. J. (1978). Anaphylactic shock and strong cardiac stimulation caused by stings of the bumble bee *Bombus terrestris* (Hymenoptera: Apidae). *N. Z. Entomol.* **6**, 385–389.

Hardouin, R. (1948). 'La vie des Abeilles Solitaires.' Librairie Gallimard, Paris.

Hermann, H. R. and Mullen, M. A. (1974). The hymenopterous poison apparatus. XI. *Xylocopa virginica* (Hymenoptera: Xylocopidae). *J. Ga. Entomol. Soc.* **9**, 246–252.

Hoffman, D. R. (1982). Allergenic cross-reactivity between honeybee and bumblebee venoms. *J. Allergy Clin. Immunol.* **69**, 139.

Mello, M. L. S. (1970). A quantitative analysis of the protein in venoms from *Apis mellifera* and *Bombus. J. Apic. Res.* **9**, 113–120.

Nakajima, T. (1979). Trace characterization of venomous animals. In 'Annual Reports on Trace Characterization' (T. Fujimaga, ed.), pp. 174–176. Scientific Grant of Education, Japan.

O'Connor, R., Rosenbrook, W. and Erikson, R. (1964). Disc electrophoresis of Hymenoptera venoms and body proteins. *Science* **145**, 1320–1321.

Piek, T., Veldsema-Currie, R. D., Spanjer, W. and Mantel, P. (1983). Acetylcholine and an unidentified muscle-contracting factor in the venom of the bumblebee, *Bombus terrestris* L. *Comp. Biochem. Physiol. C* **75C**, 351–356.

Schmidt, J. O., Blum, M. S. and Overal, W. L. (1980). Comparative lethality of venoms from stinging Hymenoptera. *Toxicon* **18**, 469–474.

Welsh, J. H. and Batty, C. S. (1963). 5-Hydroxytryptamine content of some arthropod venoms and venom containing parts. *Toxicon* **1**, 165–173.