Wheeler, W. M. (1910). 'Ants, Their Structure, Development, and Behaviour'. Columbia Univ. Press, New York.

Wheeler, W. M. (1928). A solitary wasp (*Aphilanthops frigidus* F. Smith) that provisions its nest with queen ants. *In* 'Foibles of Insects and Men' (W. N. Wheeler, ed.), Knopf, New York.

Wilbert, H. (1964). Das Ausleseverhalten von *Aphelinus semiflavus* Howard und die Abwehrreaktionen seiner Wirte (Hymenoptera: Aphelinidae). *Beitr. Entomol.* **14**, 159–221.

Willard, H. F. (1927). Parasites of the pink bollworm in Hawaii. *U. S. Dep. Agric. Tech. Bull. No. 19*, pp. 1–15.

Williams, F. X. (1919a), *Epyris extraneus* Bridwell (Bethylidae), a fossorial wasp that preys on the larva of the Tenebrionid beetle *Gonocephalum seriatum* (Boisduval). *Proc. Hawaii Entomol. Soc.* **4**, 55–63.

Williams, F. X. (1919b). A note on the habits of *Epatiothynnus opaciventris* Turner, an Australian thynnid wasp. *Psyche* **26**, 160–162.

Williams, F. X. (1919c). Philippine wasp studies. *Rep. Exp. Stn. Hawaii Sugar Plant Assoc. Stn. Entomol. Ser. Bull.* **14**.

Williams, F. X. (1927). *Euparagia scutellaris* Cresson, a masarid wasp that stores its cells with the young of a curculionid beetle. *Pan-Pac. Entomol.* **4**, 38–39.

Williams, F. X. (1928a). Studies on tropical wasps, their hosts and associates. *Bull. Exp. Sta. Hawaii Sugar Plant. Assoc. Entomol. Soc. Bull.* **19**, 1–179.

Williams, F. X. (1928b). The sphecid wasp *Podalonia violaceipennis*. *Proc. Hawaii Entomol. Soc.* **7**, 163.

Williams, F. X. (1929). Notes on the habits of the cockroach hunting wasps of the genus *Ampulex* sens. Lat., with particular reference to *Ampulex* (*Rhinopsis*) *caniculatus* Say. *Proc. Hawaii Entomol. Soc.* **7**, 315–329.

Williams, F. X. (1942). *Ampulex compressa* (Fabr.), a cockroach-hunting wasp introduced from New Caledonia into Hawaii. *Proc. Hawaii Entomol. Soc.* **9**, 221–233.

Williams, F. X. (1956). Life history studies of *Pepsis* and *Hemipepsis* wasps in California (Hymenoptera, Pompilidae). *Ann. Entomol. Soc. Am.* **49**, 447–466.

Wong, L. K. and Crowden, R. K. (1976). Preliminary studies on the mucus secretion of the wood wasp, *Sirex noctilio* F. I. Physicochemical properties. *Aust. J. Biol. Sci.* **29**, 21–32.

Woodbury, A. (1930). A note on the longevity of a paralysed orthopteran (Locustidae; Hymen: Sphecidae) *Entomol. News* **41**, 135.

Yamasaki, M. (1982). Biology of a sanitary injurious Bethylid wasp, *Cephalonomia gallicola* (Ashmead) (Hymenoptera, Bethylidae) *Jpn. J. Sanit. Zool.* **33**, 221–226.

6

Pharmacological Biochemistry of Vespid Venoms

TERUMI NAKAJIMA
Department of Analytical Chemistry
Faculty of Pharmaceutical Science
University of Tokyo
Tokyo, Japan

I. INTRODUCTION

The superfamily Vespoidea can be subdivided into the families Masaridae, Eumenidae and Vespidae. The masarid wasps differ from the other two families by the fact that many species feed on plants, and that they, like bees, feed their offspring with nectar and pollen instead of with insects. The eumenid wasps, however, prey on larvae of moths or beetles, like other solitary wasps (see Chapter 5). In contrast, the true vespid wasps kill their prey and feed their offspring with flesh of the killed prey. They are true social wasps, living in a colony founded by a queen. Some vespid wasps live in a colony with more than one queen (Evans and Eberhard, 1970; Hermann and Blum, 1981). Both workers and queens in social organizations are quite aggressive and dangerous. Nearly everybody has been stung at least once by a vespid wasp.

Vespid venoms, in general, produce prolonged pain and local edema and erythema caused by an increase in permeability of the blood vessels in the skin. The pain often continues for several hours and the itching lasts for days. Besides these direct actions of vespid wasp stings, allergic reactions have also been observed in many cases. The generalized allergic reaction may be lethal

(see Chapter 10). In addition to their actions on whole organisms, vespid venoms act kinetically on isolated smooth muscle and reduce blood pressure when they are injected intravenously. They release endogenous histamine from granulocytes such as mast cells and basophilic leucocytes; they also release catecholamines from adrenal chromaffin cells. They also cause cytolysis, including haemolysis, and chemotaxis to macrophages and polymorphonuclear leucocytes (Habermann, 1971; Edery *et al.*, 1978).

The overall action of social wasp venoms is complicated and may be described as a cumulation of active principles in the venoms. Active principles in some social wasp venoms have been isolated and investigated now by a number of scientists (Pisano, 1968; Edery *et al.*, 1978; Schmidt, 1982; Nakajima, 1984). The venoms consist of various kinds of active amines, peptides and proteins including many kinds of hydrolases (i.e. proteases, hyaluronidases, phosphatases, nucleotidases and phospholipase A), as well as allergens and high molecular weight neurotoxins.

II. ACTIVE AMINES IN VESPID VENOMS

Vespid venoms contain biologically active amines such as serotonin, histamine, tyramine and catecholamines. These are undoubtedly derived from the corresponding amino acids found in the venom-producing cells, probably by synthetic pathways comparable to those described for other animals, including vertebrates.

Although active amines appear to be the major pain-producing principles in the venoms, their concentrations differ greatly between wasp species. Table I summarizes the major amines found in the venom reservoirs for several Vespoidea together with their weight and percentage. The data suggest that most of the species of large body size, which belong to the genera *Vespa*, *Paravespula*, *Dolichovespula* and *Polistes*, contain serotonin and histamine as the major active amines in the venom. On the contrary, the solitary eumenid genera, such as *Eumenes*, *Abispa* and *Rhynchium*, as well as the small-bodied *Ropalidia*, lack serotonin, with histamine being the major amine in the venom. The venom of *Rhynchium* is also rich in active amines derived from tyrosine and dopa, being similar in this respect to the venoms of some pompilid wasps (Nakajima *et al.*, 1983). Acetylcholine has been reported to occur in the venoms of *V. crabro* (Bhoola *et al.*, 1961) and *V. orientalis* (Edery *et al.*, 1972), but there are no reports of the occurrence of this amine in other wasp species.

Polyamines including putrescine, spermidine and spermine were recently recognized as common components in the venom of Hymenoptera (Nakajima *et al.*, 1983). These amines have also been found in spider venom (Cabbiness

Table I

Active Amines in Some Vespid Venoms[a]

	Serotonin	Histamine	Tyramine	Dopamine	Noradrenaline	Adrenaline	Reference[b]
Vespa mandarinia	5.8 (56.0)	4.3 (42.0)	0.2 (2.0)	—	—	—	1
Vespa analis	3.1 (72.1)	1.1 (25.6)	0.1 (2.3)	—	—	—	1
Vespa xanthoptera	5.0 (49.0)	5.1 (50.0)	0.1 (1.0)	—	—	—	1
Vespa affinis	3.4 (56.7)	2.2 (36.7)	0.3 (5.0)	0.1 (1.7)	—	—	1
Vespa tropica	12.1 (76.6)	3.4 (21.5)	0.1 (0.6)	0.2 (1.3)	—	—	1
Vespa crabro	0.8 (38.1)	1.0 (47.6)	0.04 (1.9)	0.2 (9.5)	0.04 (1.9)	0.02 (1.0)	2,3
Vespa orientalis	8.0 (95.6)	—	—	0.2 (2.4)	0.1 (1.2)	0.05 (0.6)	4
Paravespula vulgaris	1.3 (6.0)	20.0 (92.0)	—	0.3 (1.4)	0.02 (0.1)	0.02 (0.1)	2,5
Paravespula germanica	6.2 (98.0)	—	—	0.03 (0.5)	0.07 (1.1)	0.02 (0.4)	2
Paravespula maculifrons	—	15.0 (99.4)	—	0.07 (0.5)	0.05 (0.1)	—	6
Paravespula lewisii	0.2 (18.2)	0.8 (72.7)	0.1 (9.1)	—	—	—	1
Dolichovespula arenaria	—	—	—	0.1 (96.2)	0.004 (3.8)	—	7
Dolichovespula media	0.1 (83.3)	—	—	—	—	0.02 (16.7)	2,8
Dolichovespula saxonia	1.1 (95.7)	—	—	0.02 (1.7)	0.03 (2.6)	—	2
Dolichovespula maculata	4.0 (33.2)	8.0 (66.5)	—	0.01 (0.1)	0.02 (0.2)	—	6
Polistes rothneyi	6.9 (74.8)	2.1 (22.7)	0.2 (2.2)	0.03 (0.03)	—	—	1
Polistes tepidus	15.8 (61.5)	8.9 (34.6)	0.2 (0.8)	0.8 (3.1)	—	—	1
Polistes comis	1.0 (45.5)	1.0 (45.5)	1.0 (4.5)	0.1 (4.5)	—	—	1
Polistes chinensis	0.13 (25.5)	0.3 (58.8)	0.05 (9.8)	0.03 (5.9)	—	—	1
Rhopalidia macriventris	5.8 (56.7)	3.8 (37.1)	0.5 (4.9)	0.03 (0.3)	0.1 (1.0)	—	1
Rhopalidia marginata	—	6.8 (89.5)	0.5 (6.6)	0.3 (3.9)	—	—	1
Abispa splendia	—	5.3 (86.9)	0.3 (4.9)	0.5 (8.2)	—	—	1
Eumenes arcutus	—	4.7 (92.2)	0.3 (5.9)	0.1 (1.6)	—	—	1
Eumenes latreilli	—	15.0 (96.2)	0.3 (3.8)	0.3 (3.8)	—	—	1
Rhynchium aruliferum	—	13.0 (63.4)	2.5 (12.2)	0.5 (2.5)	2.0 (9.8)	2.5 (12.2)	1
Rhynchium mirabile	—	3.3 (42.9)	1.0 (13.0)	1.0 (13.0)	1.7 (22.1)	0.7 (9.0)	1

[a]Amine contents are expressed as μg/venom sac. The values in parentheses are percentages.

[b]Key to references: 1, Nakajima *et al.* (1983); 2, Ishay *et al.* (1974); 3, Bhoola *et al.* (1961); 4, Edery *et al.* (1972); 5, Jaques and Schachter (1954); 6, Geller *et al.* (1976); 7, Owen (1971); 8, Welsh and Batty (1963).

et al., 1980) and scorpion venom (Adrujunwadker and Raguhupathi Rami Reddy, 1983). It is well known that polyamines occur in high concentrations in proliferated tissues, but it is not known whether these amines exist in the venom fluid or in the tissues of the venom reservoir. If they are present in the venom, then it is not clear whether their co-existence with other active principles of the venom might affect the overall biological action of the whole venom.

Several papers have reported that aminergic components in honey-bee venom vary quantitatively and qualitatively during the maturation of the worker bee (Owen and Bridges, 1982). A comparable maturation of the venom may occur in Vespidae, since in the oriental hornet, *Vespa orientalis*, the toxicity of the extract of the venom reservoir increases with the age of the adult wasp (Edery *et al.*, 1978).

III. PAIN-PRODUCING PEPTIDES (WASP KININS)

In 1954, Jaques and Schachter identified a pain-producing peptide in the wasp *Paravespula vulgaris* (they used the name *Vespa*). Due to the similarity in pharmacological properties of this peptide with bradykinin, a nonapeptide liberated from mammalian plasma by trypsin or certain snake venoms, this peptide was called wasp kinin. In 1968, the first structure of a wasp kinin was described. This was polisteskinin 3, an octadecapeptide isolated from a mixture of venom apparatus extracts of *Polistes exclamans*, *P. annularis* and *P. fuscatus*, in which the amino acid sequence of bradykinin was elongated at the C-terminal portion of the peptide (Pisano, 1968).

During the past 15 years, many wasp kinins have been isolated and characterized chemically, as is shown in Table II. With only a few exceptions, these wasp kinins possess the parent bradykinin sequence in their molecules. Exceptions have arisen by replacement of amino acid residues at positions 3 and 6 in the bradykinin sequence. In some of the wasp kinins of *Polistes* species, serine at position 6 is replaced by threonine, and in this respect they are like the plasmakinin of the turtle (Dunn and Packs, 1970). In other wasp kinins (i.e. that from the genus *Vespa*), replacement of proline at position 3 by hydroxyproline has been found. This has also been described for a frog skin analogue of bradykinin obtained from the frog *Heleophryne depressa* (Nakajima *et al.*, 1979). Moreover, in the kinins isolated from the venom of Polistinae, the bradykinin chain is elongated at the N-terminus, often by one or more basic amino acids. Molecular heterogeneity of wasp kinins, as is the case for polisteskinin-J, for example, is a phenomenon which has been described frequently for other natural products. It is, however, not known whether workers of *P. jadwigae* produce the serine-containing peptide or the threonine-containing peptide individually or together.

Table II

Wasp Kinins in Vespid Venoms

Species	Amino acid sequence[a]	Name of wasp kinin	Reference[b]
	1 2 3 4 5 6 7 8 9		
Vespinae	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	Bradykinin	
Vespa mandarinia	Gly-Arg-Pro-Hyp-Gly-Phe-Ser-Pro-Phe-Arg-Ile-Asp	Vespakinin-M	1
Vespa xanthoptera	Ala-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-Ile-Val	Vespakinin-X	2
Vespa analis	Gly-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-Val-Ile	Vespakinin-A	3
Vespa tropica	carbhy carbhy ' ' Gly-Arg-Pro-Hyp-Gly-Phe-Ser-Pro-Phe-Arg-Val-Val	Vespakinin-T	4
Paravespula maculifrons	Thr-Ala-Thr-Thr-Arg-Arg-Arg-Gly-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	Vespulakinin	5
Paravespula lewisii	Thr-Ala-Thr-Thr-Lys-Arg-Arg-Gly-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg ' NAcGal ' Gal	Vespulakinin-L	3
Polistinae			
Polistes fuscramatus			
Polistes exclamans	Pyr-Thr-Asn-Lys-Lys-Lys-Leu-Arg-Gly-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	Polisteskinin 3	6
Polistes annularis			
Polistes rothneyi	Ala-Arg-Arg-Pro-Pro-Gly-Phe-Thr-Pro-Phe-Arg	Polisteskinin-R	7
Polistes jadwigae	Arg-Arg-Arg-Pro-Pro-Gly-Phe-$\frac{\text{Thr}}{\text{Ser}}$-Pro-Phe-Arg Arg-Thr-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	Polisteskinin-J	3
Polistes chinensis	Ser-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	Polisteskinin-C	3

[a]carbhy, Carbohydrate.

[b]Key to references: 1, Kishimura *et al.* (1976); 2, Yasuhara *et al.* (1977); 3, Nakajima *et al.* (1984); 4, Yasuhara *et al.* (1983); 5, Yoshida, *et al.* (1976); 6, Udenfriend *et al.* (1967); 7, Watanabe *et al.* (1976).

In contrast to what has been described above for kinins of Polistinae, the kinis in the venoms of hornets (genus *Vespa*) contain a bradykinin sequence elongated at the N-terminus with one amino acid residue and at the C-terminus with two rather hydrophobic amino acid residues.

Kinins present in the venoms of *Paravespula* consist of a bradykinin molecule elongated at the N-terminus with eight amino acid residues. Moreover, this extra peptide chain contains sugar moieties connected with the hydroxyl group of the threonine residue. These peptides are the only examples of carbohydrate-containing peptides of small molecular weight. The structural differences among the wasp kinins raise interesting questions for the chemotaxonomist. Unfortunately, investigations of wasp kinins in many genera of the Vespoidea have not yet been reported, so the picture remains incomplete.

The pharmacological properties of wasp kinins have not been fully investigated because of the limited amount of natural peptides that are available. Their properties have been summarized by Edery *et al.* (1978) as follows: intravenously injected wasp kinins cause hypertension in rat, dog, rabbit and cat, and hypotension in chicken; they cause bronchoconstriction in guinea pig, and contraction of isolated smooth muscle preparations, including rat uterus, dog duodenum, rabbit jejunum, guinea pig ileum, rabbit colon and frog stomach fundus; they also cause relaxation of the rat duodenum and the hen caecum. Thus these kinins show pharmacological properties similar to that of bradykinin, kallidin (Lys-bradykinin) and Met-Lys-bradykinin isolated from mammals (Pisano, 1970, 1979). It has been reported that polisteskinin 3 was 2–20 times more potent than bradykinin in several bioassays (Pisano, 1970). The *in vivo* activity of such peptides may be enhanced by structural modifications designed to prevent attack by proteases, so as to increase their half lives. This can be achieved (a) by the elongation of the bradykinin residue at one or both sides, (b) by the introduction of one or more D-isomers in the amino acid sequence, or (c) by modification of the terminal amino or carboxyl moieties to the corresponding acyl derivates, alcohols or amides. The high activities of wasp kinins *in vivo* may be due to (a), since they are elongated amino acid or peptide chains.

In addition to the above pharmacological activities, 3×10^{-7} *M* polisteskinin 3 is known to release histamine from rat mast cells and is about 10 times more active than bradykinin in this respect (Johnson and Erdös, 1973). This polisteskinin is a basic peptide because it contains among its 18 amino acid residues six basic residues and no acidic residues, except for the C-terminal carboxylic acid. The vespulakinins are also very basic but a histamine-releasing activity of these peptides has not yet been reported.

IV. HISTAMINE-RELEASING PEPTIDES (MASTOPARANS)

A well-known principle present in honey-bee venom which liberates granules and releases histamine from cells is the mast cell degranulating peptide (MCD-peptide) (Breithaupt and Habermann, 1968) or peptide 401 (see also Chapter 7, Section V,C). Jaques and Schachter (1954) already suggested that a histamine-releasing principle like MCD-peptide might also be present in vespid venom. Moreover, some of the proteases, the phospholipases and kinins in vespid venoms were also found, either singly or in combination, to release histamine from mast cells.

Mastoparan was the first peptide with a histamine-releasing principle, isolated from the venom of *Paravespula lewisii* (Hirai *et al.*, 1978). Mastoparan is a tetradecapeptide amide without cysteine residues, and thus different from the honey-bee MCD-peptide (for MCD-peptide, see Chapter 7, Fig. 11). This peptide is rich in hydrophobic amino acids such as leucine, isoleucine and alanine and basic amino acids such as lysine. The position of lysine at positions 4, 11 and 12 are common to the group of compounds known as mastoparans. An exception is *Polistes* mastoparan, isolated from *Polistes jadwigae*. As shown in Table III, many analogues with similar structures and toxicities have been isolated from the venoms of *Vespa* species.

There is no sequence homology between the mastoparans from Vespidae and MCD-peptide from honeybees. In fact, the sequence for mastoparan resembles that of bombinin, a haemolytic peptide isolated from the skin secretions of the European frog *Bombina variegata* (Csordàs and Michl, 1970). Figure 1 illustrates the superimposed sequences of mastoparans and bombinin. At a threshold concentration of 3×10^{-7} *M* mastoparan acts on mast cells to liberate granules and release histamine (Hirai *et al.*, 1979b). This peptide also releases catecholamines and adenylic acids from adrenal chromaffin cells when applied at a concentration as low as 6×10^{-7} *M* (Kuroda *et al.*, 1980). Mastoparan-X, from the venom of *Vespa xanthoptera*, shows a haemolytic activity on rabbit erythrocytes and releases serotonin from the platelet-rich plasma of the rabbit at a concentration of 4×10^{-6} *M* (Hirai *et al.*, 1979b).

A few micrograms of mastoparan does not cause the isolated rat uterus preparation to contract nor does it elicit action in the anesthetized rat following intravenous injection. Mastoparan interacts with artificial lipid membranes constituted from lecithin and cholesterol. The peptide enhances ion permeability in the lipid bilayer, thereby increasing the membrane conductance (Okumura *et al.*, 1981). A permeability increase to inorganic ions may be the primary cause of the effect of this toxin on mast cells, chromaffin cells and platelets in releasing granules and active amines, since these cells require uptake of Ca^{2+} ions for excocytosis.

Table III

Mast Cell Degranulating Peptides (Mastoparans) in Vespid Venoms

Species	Amino acid sequence	Name of mastoparan	Reference[a]
Vespinae			
Vespa mandarinia	Ile-Asn-Leu-Lys-Ala-Ile-Ala-Ala-Leu-Ala-Lys-Lys-Leu-Leu-NH_2	Mastoparan-M	1
Vespa xanthoptera	Ile-Asn-Trp-Lys-Gly-Ile-Ala-Ala-Met-Ala-Lys-Lys-Leu-Leu-NH_2	Mastoparan-X	2
Vespa analis	Ile-Lys-Trp-Lys-Ala-Ile-Leu-Asp-Ala-Val-Lys-Lys-Val-Leu-NH_2	Mastoparan-A	*
Vespa tropica	Ile-Asn-Leu-Lys-Ala-Ile-Ala-Ala-Phe-Ala-Lys-Lys-Leu-Leu-NH_2	Mastoparan-T	3
Vespa orientalis	Ile-Asn-Leu-Lys-Ala-Ile-Ala-Ala-Leu-Val-Lys-Lys-Val-Leu-NH_2	Mastoparan II	4
Vespa crabo	Leu-Asn-Leu-Lys-Ala-Leu-Leu-Ala-Val-Ala-Lys-Lys-Ile-Leu-NH_2	Mastoparan C	5
Paravespula lewisii	Ile-Asn-Leu-Lys-Ala-Leu-Ala-Ala-Leu-Ala-Lys-Lys-Ile-Leu-NH_2	Mastoparan	6
Polistinae			
Polistes jadwigae	Val-Asp-Trp-Lys-Lys-Ile-Gly-Gln-His-Ile-Leu-Ser-Val-Leu-NH_2	*Polistes* mastoparan	6

[a]Key to references: 1, Hirai *et al.* (1981a); 2, Hirai *et al.* (1975b); 3, Yasuhara *et al.* (1983); 4, Nazimov *et al.* (1980); 5, Argiolas and Pisano)1984); 6, Hirai *et al.* (1981b); *, to be published.

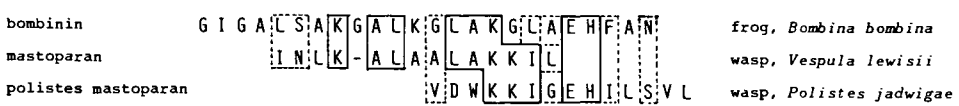


Fig. 1 Analogy between frog hemolytic peptide and mastoparans. Amino acids surrounded by dotted lines correspond to a single base change of he triplet condon of RNA.

In addition to direct action on membranes, mastoparan stimulates phospholipase A activity (Argiolas and Pisano, 1983). The peptide causes an increase in the production of arachidonic acid catalysed by phospholipase A from bee venom (12-fold increase), Eastern diamondback rattlesnake venom (15-fold) or porcine pancreas (50-fold) (Argiolas and Pisano, 1983).

High-affinity binding of mastoparans to calmodulin complexes has been determined in 0.2 *M* KCl, 1.0 m*M* $CaCl_2$, pH 7.3. The dissociation constants are 0.3 n*M* for mastoparan, 0.9 n*M* for mastoparan-X and 3.5 n*M* for *Polistes* mastoparan. These constants for mastoparan–calmodulin complexes were reported to be the smallest known for any calmodulin-binding protein or peptide (Malencik and Anderson, 1983). The interaction between mastoparan and calmodulin diminishes in the presence of 10 m*M* EDTA, suggesting that Ca^{2+} ions may be essential for the binding of mastoparan.

Conformation analysis of mastoparan in water, alcohol or in lipid vesicles has been performed by circular dichroism (CD) spectroscopy and nuclear magnetic resonance (NMR) studies (Higashijima *et al.*, 1983). Mastoparan is a random coil in water, while in methanolic solution it is an α helix. In the presence of lysophosphatidyl choline, or in a liposome of lecithin cholesterol, it takes a helical form. These results indicate that the conformation of mastoparan is governed by its microenvironment. The helix shown in Fig. 2a is an amphipathic helix in which all lysine residues are located on the same side of the axis of the helix with the hydrophobic amino acid residues in the opposite position. In a fully deuterated lipid membrane, the ϵ-amino groups of the lysine residues are considered to be in the surface of the membrane. This is indicated by the fact that the NMR-shift reagent in the outer side of the lipid vesicle affects the δ-methylene signal of the lysine residues (Wakamatsu *et al.*, 1983). Thus the mastoparans show quite interesting properties when presented in lipid membranes.

V. VESPID CHEMOTACTIC PEPTIDES

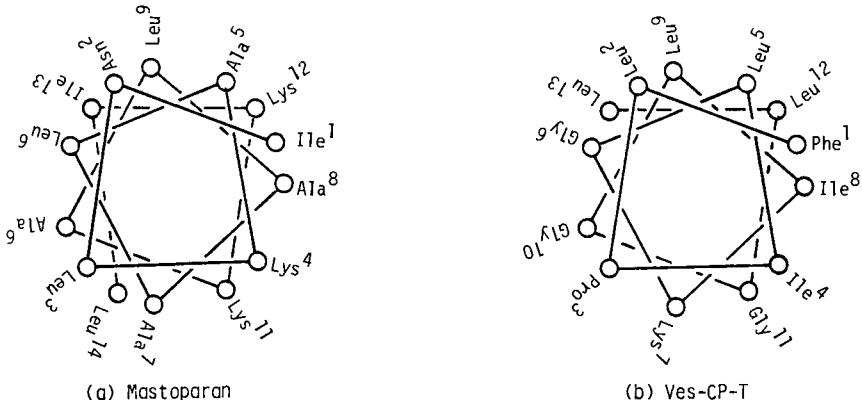
Microscopic observations of local pathological effects of wasp stings show that mild edema develops, accompanied by an inflammatory exudate containing mainly polymorphonuclear leucocytes (Sandbank *et al.*, 1971). Vespid wasps contain chemotactic principles in their venoms (Hoffman, 1978). Figure 3 shows that chromatographic studies of the venom extracts of *Vespa tropica* and *V. mandarinia* with 50% acetonitrile gave similar results using reverse phase octadecyl silicate (ODS) columns. These patterns are typical for vespid venoms. Chromatograms of extracts of the venom reservoirs

Ile^{1}-Asn^{2}-Leu^{3}-Lys^{4}-Ala^{5}-Leu^{6}-Ala^{7}-Ala^{8}-Leu^{9}-Ala^{10}-Lys^{11}-Lys^{12}-Ile^{13}-Leu^{14}-NH_2

Mastoparan

Phe^{1}-Leu^{2}-Pro^{3}-Ile^{4}-Leu^{5}-Gly^{6}-Lys^{7}-Ile^{8}-Leu^{9}-Gly^{10}-Gly^{11}-Leu^{12}-Leu^{13}-NH_2

Ves-CP-T



(a) Mastoparan (b) Ves-CP-T

Fig. 2 Amphipathic helix of mastoparan (a) and vespid chemotactic peptide (b).

of other species are also similar, with major peaks of absorption at 210 nm which correspond to wasp kinin, mastoparan and to the chemotactic peptide. In the venom of *V. tropica* the chemotactic peptide is the second major peak in the chromatogram.

The chemotactic peptides are called vespid chemotactic peptides (Ves-CPs). All Ves-CPs isolated from vespid venoms are tridecapeptide amides (Table IV). The sequences resemble those of mastoparans with regard to the chain length, richness of hydrophobic amino acids and the amidated C-termini. Ves-CP is also a random coil in water and an α helix in methanolic solution or in the presence of lysophosphatidycholine (S. Uzu *et al.*, personal communication). The helix shown in Fig. 2b is a sort of amphipathic helix in which all the hydrophobic amino acid residues, such as Leu, Ile and Phe, are located on the same side of the axis, while others, such as Gly, Pro and Lys residues, are on the opposite side. However, at comparable concentrations the Ves-CPs do not exhibit similar biological activities to those of mastoparans. At a concentration of 0.1 nmol/ml Ves-CP-T shows a 10 times higher chemotaxis to macrophages than to polymorphonuclear leucocytes. However, the chemotaxis for polymorphonuclear leucocytes also occurs to some extent through a truncated C-terminal heptapeptide amide (Yasuhara *et al.*, 1984). Ves-CPs are the first examples of chemotactic principles in

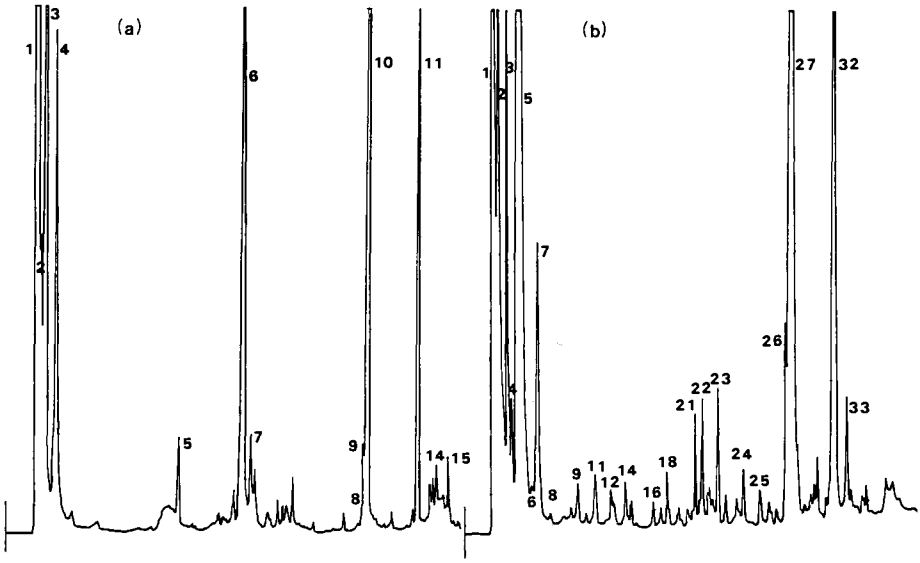


Fig. 3 Chromatographic patterns of the venom sac extracts of *Vespa mandarina* (a) and *Vespa tropica* (b). Chromatographic condition: Column: TSK LS-410 ODS, 4 mm × 300 mm. Eluent: (I) 5% CH_3CN, 10 m*M* KH_2PO_4, 50n*M* Na_2SO_4 (pH 2.5); (II) 60% CH_3CN, 10 m*M* KH_2PO_4, 50m*M* Na_2SO_4 (pH 2.5). Elution: linear gradient from 100% (I) to 100% (II) in 30 min. flow rate: 1 ml/min. column temperature: 70°C. detection: UV 210 nm. (a) Peaks 1–4: active amines (each peak is not identified); peak 6, vespakinin-M; peak 10, mastoparan-M; peak 11, Ves-CP-M. (b) Peaks 1–5: active amines (each peak is not identified); peak 21, vespakinin-T; peak 27, mastoparan-T; peak 32, Ves-CP-T.

arthropod venoms. The binding of Ves-CPs to lipid membranes is expected to be similar to that of mastoparans because of similarity in hydrophobicity. It is also noteworthy that mastoparan and Ves-CP are the major peptide components of the venom and that they do not directly produce pain, but they might play an important role in enhancing the inflammatory effects of wasp stings. Ves-CPs might be the smallest adjuvant in these venoms. A similar peptide, crabrolin, was isolated from the venom of the European hornet, *Vespa crabro* (Argiolas and Pisano, 1984). The peptide is also a tridecapeptide amide with a new amino acid sequence:

Phe-Leu-Pro-Leu-Ile-Leu-Arg-Lys-Ile-Val-Thr-Ala-Leu-NH_2

VI. ALLERGENS

In addition to the direct toxic action of many enzymes present in vespid venoms, these enzymes have been recognized as allergens (Hoffman, 1978;

Table IV

Chemotactic Peptides in Vespid Venoms

Species	Amino acid sequence	Name of peptide	Reference[a]
Vespa tropica	Phe-Leu-Pro-Ile-Leu-Gly-Lys-Ile-Leu-Gly-Gly-Leu-Leu-NH_2	Ves-CP-T	1
Vespa mandarinia	Phe-Leu-Pro-Ile-Ile-Gly-Lys-Leu-Leu-Ser-Gly-Leu-Leu-NH_2	Ves-CP-M	2
Vespa analis	Phe-Leu-Pro-Met-Ile-Ala-Lys-Leu-Leu-Gly-Gly-Leu-Leu-NH_2	Ves-CP-A	*
Vespa xanthoptera	Phe-Leu-Pro-Ile-Ile-Ala-Lys-Leu-Leu-Gly-Gly-Leu-Leu-NH_2	Ves-CP-X	*
Paravespula lewisii	Phe-Leu-Pro-Ile-Ile-Ala-Lys-Leu-Val-Ser-Gly-Leu-Leu-NH_2	Ves-CP-L	*
Related peptides to Ves-CPs			
Vespa crabro	Phe-Leu-Pro-Leu-Ile-Leu-Arg-Lys-Ile-Val-Thr-Ala-Leu-NH_2	Crabrolin	3
Vespa orientalis	Phe-Leu-Pro-Leu-Ile-Leu-Gly-Lys-Leu-Val-Lys-Gly-Leu-Leu-NH_2	HR-II	4
Icaria sp.	Ile-Val-Pro-Phe-Leu-Gly-Pro-Leu-Leu-Gly-Leu-Leu-Thr-NH_2		*

[a]Key to references: 1, Nakajima *et al.* (1984); 2, Yashuhara *et al.* (1984); 3, Argiolas and Pisano (1984); 4, Miroshnikov *et al.* (1981); *, to be published.

King *et al.*, 1978) (see also Chapter 10). Be fractionation of the venom of an unidentified yellow jacket, Hoffman (1978) demonstrated multiple allergens. RAST (radioallergosorbent test) positive sera for the crude venom were examined against these fractionized venom components. The allergens eluted in the fractions corresponded to the enzyme activities of acid phosphatase, hyaluronidase and phospholipase together with other nonenzymatic fractions of smaller molecular weight. Hoffman (1981) reported cross-reactivity of human IgE antibodies between venoms from yellow jackets (Vespidae) and paper wasps (Polistinae). About 62% of individuals sensitive to the venom of vespid wasps reacted to more than one of the three venoms from yellow jacket, yellow hornet and white-faced hornet by RAST, and 79% of paper wasp (Polistinae)-reactive individuals also reacted to venoms from yellow jackets (Vespidae).

King *et al.* (1978) have isolated nonenzymatic protein allergens from the venom of *Dolichovespula maculata*, *D. arenaria* and yellow jackets of the genus *Paravespula* (mixture of *P. maculifrons*, *P. vulgaris* and *P. germanica*). Those venoms were separated by Sephadex G-100 chromatography. The nonenzymatic allergens are called the antigens 5, after the fraction number of the chromatography. Antigens 5 do not exhibit any of the known enzyme activities found in vespid venom and are reported to be basic proteins with a molecular weight of 25,000. Judging from their amino acid composition, all of the antigens 5 are analogous proteins. They have been reported to be the most active allergenic substances present in these venoms, more active than the enzymes hyaluronidase, phospholipase and acid phosphatase. Einarsson and Renck (1984) have reported a rapid and precise separation method of venomous enzymes in honey-bee and vespid venoms, including the antigens 5, by high performance liquid chromatography using a cation exchange column.

VII. NEUROTOXINS

Neurotoxic components are present not only in venoms of paralysing solitary wasps (see Chapter 5) but also in the venoms of the Vespidae and in bee venom (see Chapter 7). In 1976, Kawai and Hori for the first time reported the presence of neurotoxic components in the Sephadex-fractionated venom of *Vespa mandarinia*, *V. xanthoptera* and *V. analis insularis*. These neurotoxins block neuromuscular transmission in the walking legs of lobster. The partially purified toxin of *V. analis insularis* is also neurotoxic to crayfish (Hori *et al.*, 1977), and the crude venom of *Dolichovespula* induces short-term paralysis when injected near a ganglion in individuals of the same species (Schmidt and Blum, 1979).

The most purified vespid neurotoxin, mandaratoxin, has been isolated from the venom of *Vespa mandarinia* (Abe *et al.*, 1982). This toxin was purified by successive chromatographic separations with Sephadex G-50 and CM-Sephadex C-50 columns. The purified toxin showed a single band when subjected to the conventional disc gel electrophoresis and sodium docecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). Mandarotoxin is a basic protein with an estimated molecular weight of 19,000 to 21,000 using the gel permeation method, SDS-PAGE and amino acid analysis. No amino sugars have been detected in the toxin. Mandarotoxin does not show enzymatic activity of the esterase, phosphatase phospholipase or protease type.

When applied to the neuromuscular junctions of the lobster, mandarotoxin increases initially the amplitude of excitatory postsynaptic potentials (EPSPs) within a few minutes, but the EPSP then declines and finally disappears. The action of mandarotoxin is irreversible. The active site of the toxin is considered by Abe *et al.* (1982) to be presynaptic because the resting conductance of the muscle fibre is not affected and because by using intraaxonal recording near the nerve terminal it was found that mandarotoxin blocks the nerve terminal potential.

Details of the structures of the antigens 5 and mandaratoxin are not yet available. However, these proteins are commonly rich in Asn, Gln, Gly and Lys residues, and poor in Met, Phe, Trp and Arg residues. In addition, the other amino acid residues in these proteins show similar compositions. Accordingly, as shown in Fig. 4, the ratios of their amino acid compositions illustrated by radar charts are strikingly similar. This may also be true for their molecular size and basic character. There might be a chemotaxonomical correlation on a molecular base among these high molecular weight toxins.

A similar toxic component may be present in the venom of the *Vespa tropica* (Gawade, 1983). However, in insect muscle fibres this venom also causes a large and irreversible increase in membrane conductance (T. Piek and S. P. Gawade, personal communication). The venom contains histamine- and serotonin-like activity, and contains ~ 1 μg glutamate per venom reservoir (R.D.. Veldsema-Currie, personal communication). These findings indicate further research to justify the conclusions by Gawade (1983). Another nonenzymatic protein which has central nervous system sites of action has been reported in the venom of *V. orientalis* (Ishay, 1979). Interestingly, this toxin was purified by an affinity column bound with anti-*Physalia physalis* (jelly fish) toxin antibody (Russo *et al.*, 1983). The purified toxin contained a major component the molecular weight of which was estimated to be 43,000 using SDS-PAGE. The toxin was reported to be an anticholinergic agent. Its toxic effects could be counteracted by atropine or heparin.

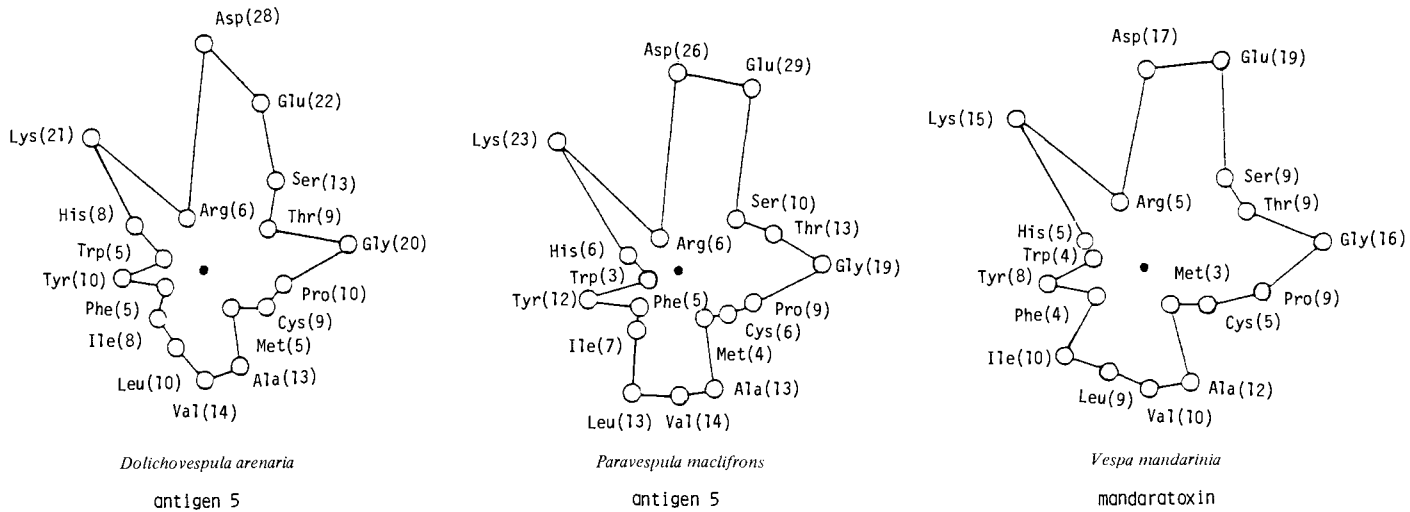


Fig. 4 Amino acid compositions of the protein toxin in vespid venoms. The number in parentheses is the nearest integer of amino acid residues.

Two kinds of paralysing toxin of high molecular weight were recently isolated from the venom of a braconid wasp (Visser *et al.*, 1983; see also Chapter 5).

VIII. CONCLUSION

During the past several years, rapid microtechniques for biochemical analysis and characterization have provided us with much information on bioactive agents which are present in nature in minute quantities. As a result of the application of these methods in insect biochemistry, many kinds of pheromones, defense substances, hormones and venomous principles have been characterized. In 1967, it required more than 6000 wasps and took almost 3 years to obtain an entire sequence of polisteskinin, while in 1982, one venom reservoir was sufficient to isolate and characterize three kinds of peptides, vespakinin-T, mastoparan-T and Ves-CP-T, within a year. By using rapid microanalytical methods it has become much easier to investigate the venoms of, for example, solitary wasps or even those of rare species of social wasps which are difficult to obtain in large numbers.

Venomous principles present in the venoms of vespid wasps and honey-bees consist of various kinds of active amines, peptides and proteins. The immediate pain we feel after a sting of a vespid wasp may be caused mainly by large amounts of serotonin, wasp kinin and protease. These substances differ completely from the pain-producing principles present in honey-bee venom (Chapter 7). Moreover, mastoparan, vespid chemotactic peptides and mandarotoxin are structurally different from apamin, melittin or MCD-peptide, found in honey-bee venom. Yet, their sites and modes of actions are similar.

Toxins found both in vespid and honey-bee venoms interact generally with the target cell membrane causing microenvironmental changes of ionic conductance. These toxins could become useful tools for providing more information on the molecular pharmacology of ion channels of mammalian cells.

REFERENCES

Abe, T., Kawai, N. and Niwa, A. (1982). Purification and properties of a presynaptically acting neurotoxin, mandaratoxin, from hornet (*Vespa mandarinia*). *Biochemistry* **21**, 1693–1697.

Adrjunwadker, A. V. and Raguhupathi Rami Reddy, S. (1983). Spermidine in the venom of scorpion, *Palamneus phipsoni*. *Toxicon* **21**, 321–325.

Argiolas, A. and Pisano, J. J. (1983). Facilitation of phospholypase A_2 activity by mastoparans, a new class of mast cell degranulating peptides from wasp venom. *J. Biol. Chem.* **258**, 13697–13702.

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

Bhoola, K. D., Calle, J. D. and Schachter, M. (1961). Identification of acetylcholine, 5-hydroxytryptamine, histamine and a new kinin in hornet venom (*V. crabro*). *J. Physiol. (London)* **159**, 167–182.

Breithaupt, H. and Habermann, E. (1968). Peptid (MCD-Peptid) aus Bienegift: Isolierung, biochemische und pharmakologische Eigenschaften. *Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol.* **261**, 252–270.

Cabbiness, S. G., Gehrke, C. W., Kuo, K. C., Chan, T. K., Hall, J. E., Hudiburg, S. A. and Odell, G. V. (1980). Polyamines in some tarantula venoms. *Toxicon* **18**, 681–683.

Csordàs, A. and Michl, H. (1970). Isolierung und Strukturraufklärung eines hamolytisch wirkenden Polypepties aus dem Abwehrsekret europäischer Unken. *Monatsh. Chem.* **101**, 182–189.

Dunn, R. S. and Parks, A. M. (1970). A new plasmakinin in the turtle, *Pseudemys elegans*. *Experientia* **26**, 1220–1221.

Edery, H., Ishay, J., Lass, I. and Gitter, S. (1972). Pharmacological activity of oriental hornet (*Vespa orientalis*) venom. *Toxicon* **10**, 13–23.

Edery, H., Ishay, J., Gitter, S. and Joshua, H. (1978). Venoms of Vespidae. In 'Arthropod Venoms' (S. Bettini, ed.), pp. 691–771. Springer-Verlag, Berlin and New York.

Einarsson, R. and Renck, B. (1984). Ion-exchange chromatographic characterization of stinging insect vespid venoms. *Toxicon* **22**, 154–160.

Evans, H. E. and Eberhard, M. J. W. (1970). 'The Wasps'. Univ. of Michigan Press, Ann Arbor.

Gawade, S. P. (1983). The effect of venom from the Indian tropical wasp *Vespa tropica* on nerve-muscle preparations from *Drosophila larvae*. *Toxicon* **21**, 882–886.

Gellar, R. G., Yoshida, H., Beaven, M. A., Horakova, Z., Atkins, F. L., Yamabe, H. and Pisano, J. J. (1976). Pharmacologically active substances in venoms of the bald faced hornet *Vespula (Dolichovespula) maculata*, and the yellow jacket *Vespula (Paravespula) maculifrons*. *Toxicon* **14**, 27–33.

Habermann, E. (1971). Chemistry, pharmacology and toxicology of bee, wasp and hornet venoms. In 'Venomous Animals and Their Venoms' (W. Bücherl and E. E. Buckley, eds.), Vol. III, pp. 61–93. Academic Press, New York.

Hermann, H. R. and Blum, M. S. (1981). Defensive mechanisms in the social Hymenoptera. In 'Social Insects' (H. R. Hermann, ed.), Vol. 2, pp. 77–197. Academic Press, New York.

Higashijima, T., Wakamatsu, K., Takemitsu, M., Fujino, M., Nakajima, T. and Miyazawa, T. (1983). Conformational change of mastoparan from wasp venom on binding with phospholipid membrane. *FEBS Lett.* **152**, 227–230.

Hirai, Y., Yasuhara, T., Yoshida, H. and Nakajima, T. (1978). A new mast cell degranulating peptide "mastoparan" in the venom of *Vespula lewisii*. In 'Peptide Chemistry 1977' (S. Shiba, ed.), pp. 155–160. Protein Research Foundation, Osaka.

Hirai, Y., Kuwada,, M., Yasuhara, T., Yoshida, H. and Nakajima, T. (1979a). A new mast cell degranulating peptide (II). Homologous to mastoparan in the venom of Japanese hornet (*Vespa xanthoptera*). *Chem. Pharm. Bull.* **27**, 1495–1496.

Hirai, Y., Yasuhara, T., Yoshida, H., Nakajima, T., Fujino, M. and Kitada, C. (1979b). A new mast cell degranulating peptide "mastoparan" in the venom of *Vespula lewisii*. *Chem. Pharm. Bull.* **27**, 1942–1944.

Hirai, Y., Yasuhara, T., Yoshida, H. and Nakajima, T. (1981a). A new mast cell degranulating peptide, mastoparan-M, in the venom of hornet *Vespa mandarinia*. *Biomed. Res.* **2**, 447–449.

Hirai, Y., Yasuhara, T., Yoshida, H. and Nakajima, T. (1981b). A new mast cell degranulating peptide, Polistes-mastoparan, in the venom of *Polistes jadwigae*. *Biomed. Res.* **1**, 185–187.

Hoffman, D. R. (1978). Allergens in Hymenoptera venom. V. Identification of some of the enzymes and demonstration of multiple allergens in yellow jacket venom. *Ann. Allergy* **40**, 171–176.

Hoffman, D. R. (1981). Allergens in Hymenoptera venom. VI. Cross reactivity of human IgE antibodies to the three vespid venoms and between vespid and paper wasp venoms. *Ann. Allergy* **46**, 304–309.

Hori, S., Kawai, N., Niwa, A. and Ohtani, S. (1977). Separation of neurotoxins from hornet (*Vespa insularis*) venom and their actions on crustacean neuromuscular transmission. *J. Neurochem.* **28**, 1183–1188.

Ishay, J. S. (1979). Anticholinesterase-like activity by oriental hornet (*Vespa orientalis*) venom and venom sac extract. *Experientia* **35**, 636–639.

Ishay, J. S., Abraham, Z., Grunfeld, y. and Gitter, S. (1974). Catecholamines in social wasps. *Comp. Biochem. Physiol. A* **48A**, 369–373.

Jaques, R. and Schachter, M. (1954). The presence of histamine, 5-hydroxytryptamine and a potent slow contracting substance in wasp venom. *Br. J. Pharmacol.* **9**, 53–57.

Johnson, A. R. and Erdös, E. G. (1973). Release of histamine from mast cells by vasoactive peptides. *Proc. Soc. Exp. Biol. Med.* **142**, 1252–1256.

Kawai, N. and Hori, S. (1976). Effect of hornet venom on crustacean neuromuscular junctions. In 'Animal, Plant, and Microbial Toxins' (A. Ohsaka, K. Hayashi and Y. Sawai, eds.), Vol. 2, pp. 309–318. Plenum, New York.

King, T. P., Sobotka, A. K., Alagon, A., Kochoumian, L. and Lichtenstein, L. M. (1978). Protein allergens of white-faced hornet, yellow hornet and yellow jacket venoms. *Biochemistry* **17**, 5165–5177.

Kishimura, H., Yasuhara, T., Yoshida, H. and Nakajima, T. (1976). Vespakinin-M, a novel bradykinin analogue containing hydroxyproline, in the venom of *Vespa mandarinia* Smith. *Chem. Pharm. Bull.* **24**, 2896–2897.

Kuroda, Y., Yoshioka, M., Kumakura, K., Kobayashi, K. and Nakajima, T. (1980). Effects of peptides on the release of catecholamines and adenine nucleotides from cultured adrenal chromaffin cells. *Proc. Jpn. Acad., Ser. B* **56** 660–664.

Malencik, D. A. and Anderson, S. R. (1983). High affinity binding of the mastoparans by calmodulin. *Biochem. Biophys. Res. Commun.* **114**, 50–56.

Miroshnikov, A. I., Snezhkova, L. G., Nazimov, I. V., Reshetova, O. I., Rozynov, B. V. and Gushchin, I. S. (1981). Structure and properties of histamine-releasing peptides from the venom of the hornet *Vespa orientalis*. *Soviet J. Bioorg. Chem.* **7**, 787–796.

Nakajima, T. (1984). Biochemistry of vespid venom. In 'Encyclopedic Handbook of Animal Toxins' (A. A. Tu, ed.), Vol. 3, pp. 109–133. Dekker, New York.

Nakajima, T., Yasuhara, T., Erspamer, G. F. and Visser, J. (1979). Occurrence of Hyp^3-bradykinin in methanol extracts of the skin of the South African leptodactylid from *Heleophryne purcelli*. *Experientia* **35**, 1133.

Nakajima, T., Yasuhara, T., Yoshida, N., Takemoto, Y., Shinonaga, S., Kano, R. and Yoshida, H. (1983). The pattern analysis of biologically active amines in some Hymenopteran vemons by high performance liquid chromatography. *Jpn. J. Sanit. Zool.* **34**, 67–71.

Nakajima, T., Yasuhara, T., Yoshida, H., Ueno, Y., Ohtsuka, C., Hamamoto, M., Nobumori, M. and Hirai, Y. (1984). Waspkinins in some Japanese wasps (Vespidae, Hymenoptera). *Jpn. J. Sanit. Zool.* **35**, 139–147.

Nazimov, I. V., Snezhova, L. G. and Miroshnikv, A. I. (1980). Structure and properties of mastoparan II. An oligopeptide from the venom of *Vespa orientalis* hornet. *Proc. 3rd Symp. Chem. Pept. Proteins*. USSR-FRG.

Okumura, K., Inui, K., Hirai, Y. and Nakajima, T. (1981). The effect of mastoparan of ion movement in black lipid membrane. *Biomed. Res.* **2**, 450–452.

Owen, M. D. (1971). Insect venoms: Identification of dopamine and noradrenaline in wasp and bee stings. *Experientia* **27**, 544–545.

Owen, M. D. and Bridges, A. R. (1982). Catecholamines in honey bee (*Apis mellifera* L.) and *Polistes humilis* (Hymenoptera, Vespidae). *Toxicon* **20**, 1075–1084.

Pisano, J. J. (1968). Vasoactive peptides in venoms. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **27**, 58–62.

Pisano, J. J. (1970). Kinins in non-mammalian origin. *Hand. Exp. Pharmacol.* **25**, 589–596.

Pisano, J. J. (1979). Kinins in nature. *Hand. Exp. Pharmacol., Suppl.* **25**, 273–285.

Russo, A. J., Cobbs, C. S., Ishay, J. S., Calton, G. J. and Burnett, J.W. (1983). Isolation of a lethal factor from the venom of *Vespa orientalis* (oriental hornet) by affinity chromatography using cross reactive monoclonal antibody. *Toxicon* **21**, 166–169.

Sandbank, U., Ishay, J. and Gitter, S. (1971). Mitochondrial changes in the guinea pig muscle after envenomation wth *Vespa orientalis* venom. *Experientia* **27**, 303–304.

Schmidt, J. O. (1982). Biochemistry of insect venoms. *Annu. Rev. Entomol.* **27**, 339–368.

Schmidt, J. O. and Blum, M. S. (1979). Toxicity of *Dolichovespula maculata* venom. *Toxicon* **17**, 645–648.

Udenfriend, S., Nakajima, T. and Pisano, J. J. (1967). Structure of the major kinin in wasp (*Polistes*) venom. *Proc. Int. Cong. Biochem., 7th*, VIII-4, p. 501.

Visser, B. J., Labruyère, W. T., Spanjer, W. and Piek, T. (1983). Characterization of two paralysing protein toxins (A-MTX and B-MTX), isolated from a homogenate of the wasp *Microbracon hebetor* (Say). *Comp. Biochem. Physiol. B* **75B**, 523–530.

Wakamatsu, K., Higashijima, T., Fujino, M., Nakajima, T. and Miyazawa, T. (1983). Transferred NOE analysis of conformations of peptides as bound to membrane bilayer of phospholipid; mastoparan-X. *FEBS Lett.* **162**, 123–126.

Watanabe, M., Yasuhara, T. and Nakajima, T. (1976). Occurrence of Thr^6-bradykinin and its analogous peptide in the venom of *Polistes rothneyi iwatai* V. der Vecht. In 'Animal, Plant, and Microbial Toxins' (A. Ohosaka, K. Hayashi and Y. Sawai, eds.), Vol. II, pp. 105–112. Plenum, New York.

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

Yasuhara, T., Yoshida, H. and Nakajima, T. (1977). Chemical investigation of hornet (*Vespa xanthoptera* Cameron) venom. The structure of a new bradykinin analogue "Vespakinin-X" *Chem. Pharm. Bull.* **25**, 935–941.

Yasuhara, T., Nakajima, T. and Erspamer, V. (1983). Isolation and sequence analysis of peptide in the picomolar level. In 'Peptide Chemistry 1982' (S. Sakakibara, ed.), pp. 213–218. Protein Research Foundation, Osaka.

Yasuhara, T., Nakajima, T., Fukuda, K., Tsukamoto, Y., Mori, M., Kitada, C. and Fujino, M. (1984). Structure and activity of chemotactic peptide from the venom sac of *Vespinae*. In 'Peptide Chemistry 1983' (E. Munekata, ed.), pp. 185–190. Protein Research Foundation, Osaka.

Yoshida, H., Gellar, R. G. and Pisano, J. J. (1976). Vespulakinins: New carbohydrate-containing bradykinin derivates. *Biochemistry* **15**, 61–64.