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Chemistry of Animal Venoms

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Introduction

In recent years the field of research on animal venoms is in a stage of a constant expansion and it has been claimed[1] that about 10,000 papers are published every year on this subject. This increase in the scientific interest for this field represents a certain essential change in the applicative as well as theoretical attitude to the subject.

In the past, the medical aspect of venoms served not only as the primary motivation but as the sole purpose of work. However present toxinology passes beyond the medical scope and venoms are used as: tools in the study of essential physiological processes and phenomena, potential sources for new therapeutic agents and as devices for the study of zooecological relationships, behaviour as well as taxonomy of venomous animals.

In other words, the chemical work on venoms is not solely concentrated in the search, isolation and structure of compounds directly involved in the lethal effects to the human being, but also in many other substances possessing a wide range of biological activities. In this review dealing with the chemistry of animal venoms, an attempt was made to present the subject in its broader conception.

Composition

Surveying a broad theme one is often obliged to make the choice between a superficial but complete coverage of the whole subject or detailed presentation of only a part of it. In order to satisfy both contradictory tendencies we shall introduce the majority of the information concerning chemistry and composition of venoms in a summarized form of a table (Table I), serving as background for a more emphasized presentation of several selected aspects.

In Table I, the venoms are classified according to the common taxonomical order of the animal kingdom[2]. The lethal potency of certain venoms and toxins is presented in Table I in a separate column, expressed by the common parameter of mice LD_{50}, thus enabling a convenient comparison. The structure of certain toxins is presentend in Figure 1. Surveying Table I, one is impressed by the wide range and diversity of groups and species of venomous animals starting from unicellular organisms up to mammals including the main phyla of marine and terrestrial animals. This is accompanied by the heterogeneity in the anatomical devices for the formation and release of toxic substances, starting from the simplest forms of storage in the body tissues as in case of poisonous fishes[3] or certain beetles[4] up to the highly sophisticated stinging apparatuses of certain arthropods[5]. Highly diverse are also the pathological effects caused by different venoms, starting with irritant repulsive secretions like those of certain arthropods[6], pain producing mixtures as found in certain wasps[7,8] and marine organisms[9], necrotic histolytic effects like those of crotalid and viperid venom[10,90] up to the potent paralytic effects caused by elapid snakes and scorpion venoms[11,12].

This diversity in action is the expression of the tremendous diversity in the chemical composition. As demonstrated in Table I, animal venoms include series of simple aliphatic materials in the form of common hydrocarbons, alcohols, aldehydes, ketones, esters and carboxylic acids as well as cyclic compounds such as phenols and quinones, all typical to defensive secretions of arthropods[4], tryptamine and phenylthiamine bases make up the main pain-producing factors; complexed alkaloids of triterpenic-steroidic nature including their glycosidic derivatives, typical for amphibian venoms; heterogenous series of proteins starting with short peptides as those present in amphibian skin secretions, low molecular basic stable proteins typical for elapid snake and scorpion venoms, high molecular neurotoxic labile proteins as typical for certain venomous fishes up to a glycolupoproteic complexes as represented by the toxic substance, prymnesin, secreted by a dinoflagellate.

These diverse compounds never appear as sole venom constituents but rather as parts of highly heterogenic

Table I. Composition of animal venoms

Animal group	Venom composition	Mice LD_{50} (μg/kg)
Protista (unicellular organisms) Order: Dinoflagellata	A) Saxitoxin, m.w. 372, $C_{10}H_{17}N_7O_4$-2 HCl, water soluble dibasic salt (pKa 8.2, 11.5). Structure supposed as perhydropurine nucleus incorporated by two guanidinium moieties (Figure 1[177–180,9]). B) Gymnodinium toxin-partly purified $C_{25}H_{42}O_8PO_2$ m.w. 468[182,183]. Prymnesin partly purified, a complexed lipo-gluco-protein. (20.4% protein, 0.47% phosphate, 10–12% sugar, and complex of fatty acids[184].	A) i.v. 3.4 i.p. 10.0 p.o. 263.0[180] B) i.v. 500[182]
Porifera (Sponges)	Toxic components not identified. Acetylcholine, histimine purines, sterols etc. were revealed among 36 different compounds[185]. Heat stable not identified principle extracted by organic solvents[186].	
Coelenterata	Lethal and paralyzing effects are due to proteins: Jelly fishes[187–190], m.w. 150,000 and 70,000. Hydrozoans-[181,193]. Sea anemones – C) Basic proteins, m.w. 13,000[191] and 10,000–15,000[192]. Phospholipase A and B[181]. Anticoagulant non dialysable heat resistant factor[194].	C) i.v. 33[191]
Nemertea Order Hoplonemertini	Anabaseine-2-(3-pyridyl) 3,4,5,6 Tetrahydropyridine (Figure 1[195–197]).	
Arthropoda Class Crustacea	Toxic crabs[198,199] were found to contain saxitoxin[198–201]. Isopoda – repulsive secretions containing a mixture of alcohols (hexan-1-ol, octan-1-ol etc.)[4].	
Class Myriapoda Subclass Diplopoda	Alkaloids–including glomerin[4]. Quinones–several derivatives of 1,4-benzoquinone (Figure 1[202,6,159]). Phenolic – secretions-*p*-cresol[4]. Cyanogenic mechanisms – hydro cyanic acid, benzaldehyde, mandelonitrile[204,17].	
Subclass Chilopoda	5-HT[4]. Hydrocyanic acid[205]. Proteolytic enzymes[206]. Benzoquinones[207].	
Class Insecta Orders Blattodea	Alcohols, unsaturated aldehydes[6,208]. Carboxylic acids[4]. Quinones[193]	
Isoptera	Quinones[4].	
Dermaptera Phasmida	Aldehydes[4].	
Orthoptera	Steroids (calotropin, calactin) and histamine[4].	
Heteroptera	Integumantal secretions hydrocarbons (n-undecane, n-dodecane etc.). Alcohols, Aldehydes (saturated, unsaturated and keto)[4]. Secretions from stinging apparatuses: Bed bugs-anticoagulant enzymes, hyaluronidase, histamine[203,209]. D) Reduviid bugs-proteic direct lytic factor, enzymes: gelatinase, esterase, hyaluronidase, anticoagulant[210,211] trypsin like and weak PhA activity[212], Neurotoxic factors[210,211].	D) i.v. 1000[211]
Lepidoptera	Material stored in body tissues, Cardiac glycosides[213], aristolachic acid, hydrocyanic acid[214], choline derivatives, histamine, alkaloids[4]. Barbed setae-urticating substance supposed to be a protein[215].	
Coleoptera	Integumental secretions: hydrocarbones[4], aromatic aldehydes, carboxylic acids, ester, phenols[4], quinones[216,13]. Toxins in body fluids: Cantharidin (lactone anhydride $C_{10}H_{12}O_4$)[218,220] Figure 1. E) Pederin (amide[219–220]). Toxic proteins[221–222].	E) i.p. 140[4]
Hymenoptera Family Formicidae	Iridomyrmecine-lactone of α(2-hydroxymethyl-3-methyl-cyclopentyl)-propionic acid (Figure 1[223,202]). Dendrolasin (β-4:8-dimethyl-anona 3,7-dienyl)furan (Figure 1[218]). Enzymes: Hyaluronidase, PhA, Histamine releasing factor[4,225,226]. Alkaloids (in fire ants) 2,6-disubstituted piperidines[227].	
Family Apidae	Low molecular basic proteins: Melittin (m.w. 2840)[228]. Apamin (m.w. 2038)[229]. Mast cell degranulating (MCD) – peptide (m.w. 2593)[230]. Enzymes-PhA – m.w. 19,000, acidic protein[231,232], hyaluronidase. Histamine[233].	(a) C.V., i.v. 6000 (b) i.v. 4000 (c) i.v. 4000 (d) i.v. 40,000 (e) i.v. 7500[235]
Family Vespidae	Biogenic amines: histamine, 5-HT, adrenaline, acetylcholine, kinein. Enzymes: protease, hyaluronidase, histamine releasing factor[234,235a,235].	
Class Arachnida, Order Scorpiones	Toxic proteins: Low molecular (6500–9000) basic, single chained cross linked by 4 disulfide bridges[133–140,147–151]. Presence of proteic toxins selectively active on insects[166–172] as well as crustaceans[166,173]. Enzymes: proteinase[236], PhA[236a,237], direct lytic activity[51], hyaluronidase[239,50], 5-HT[241,242].	(f) C.V., s.c. 420.0 (g) Txn. s.c. 9.0 (h) C.V., s.c. 359.5 (i) Txn. s.c. 25.0[147]

Table I (continued)

Animal group	Venom composition	Mice LD_{50} (μg/kg)
Order Araneae Theridiidae	Neurotoxic proteins specifically active on mammals and insects[244–246]. Hyaluronidase, 5-HT[246].	(j) C.V., i.p. 590[243]
Sicariidae and Theraphosidae	Proteic toxic factors[247,248]. Enzymes: hyaluronidase[248], phosphodiesterase[249], proteolytic enzyme[250,251], DNAse[251].	
Order Phalangida	Repulsive secretions: dimethyl and trimethyl 1,4-benzoquinones[44,253].	
Order Pedipalpida	Repulsive secretions: acetic acid, caprylic acid[252,45].	
Order Acarina (Genus: *Ixodes*)	Toxic fraction with antigenic properties resistant to proteolytic enzymes[254].	
Molusca Class Gastropoda	Presence of Saxitoxin[2,9]. In conidae: toxic, high molecular protein[255,256]. Enzymes proteinase[257]. In *Neptunea*: Biogenic amines; histamine, choline[255,256]. 5-HT[260]. In *Murex*: F) Murexine – $C_{11}H_{18}O_3N_3$, structure: β (imidazolyl) 4-acrylcholine (Figure 1[261]).	F) i.v. 8,400[261]
Class Cephalopoda	Biogenic amines: Tyramine, adrenaline, 5-HT, histamine[262,264]. Enzymes: Protease[62], hyaluronidase mucinolytic enzyme[263], dopadecarboxylase[264]. Toxic peptides: Eledoisin-endecapeptide[265]. Toxic proteins: Cephalotoxin, m.w.30,000–70,000 (isolated from *Sepia*[266]). Maculotoxin, m.w. 540, stable highly polar, chemically similar to tetrodotoxin[267].	
Echinodermata Class Asteroidea (starfish)	Saponins[268–270]. Sterols[271]. Homarine (n-methyl picolinic acid)[9]. Saxitoxin[322].	
Class Echinoidea (sea urchins)	Toxic proteins[272–274]. Histamine releasing, high molecular protein[273]. Saponins[275].	
Class Holothurioidea (sea cucumbers)	G) Holothurins, glucosides of tetracyclic triterpens the aglycon holothurinogenin (Δ 7.9 [11]-holostadien 3-β-17-α-diol). The sugar: a chain of 4 units to which an anionic locus in the form of half esterified sulfate is substituted (Figure 1[276–279]). Stichoposides – an additional group of triterpene glycosides[280].	G) i.v. 9000[9]
Vertebrata Class: Pisces (fish)	Toxins from body tissues: H) Ciguatoxin, partly purified[281]. $C_{35}H_{65}NO_8$ containing quarternary nitrogen atom, several hydroxyl groups and cyclopentanone moiety[282]. I) Tetrodotoxin, $C_{11}H_{17}O_8N_3$, 2-amino-6-hydroxymethyl-8-quinozolinol (Figure 1[283–285,79–82]). Toxins from skin secretions: Grammistins (from soap fish), polypeptides with an unknown moiety[287,288]. Proteic toxins from the soap fish[289], from a flat fish[290,291]. K) Pahutoxin from box fishes, $C_{23}H_{46}NO_4Cl$. Structure: choline chloride ester of 3-acetoxy hexadecanoic acid (Figure 1[292]). Toxins from venomous apparatuses: Toxic proteins in sting rays, highly labile, m.w. about 100,000[293] in weever fishes[295,296], in scorpion fishes[297–299]. Enzymes: 5-nucleotidase, phosphodiesterase[2]. Biogenic amines: 5-HT, histamine, adrenaline and noradrenaline[2,9,300].	H) i.v. 80[2] I) i.v. 11[286] K) i.v. 200,000[292] (k) C.V., i.v. 200[294] (l) i.c. 900[299]
Class: Amphibia Order: Urodela	Skin secretions: L) Alkaloids-Samandarine, $C_{19}H_{31}NO_2$, saturated secondary amine with a secondary hydroxy group (Figure 1), about 10 derivatives were isolated[301–304]. Tetrodotoxin-found in Taricha newts[305,306]. Peptides hemolytic in action. Proteins, lethal and hemolytic[307].	L) 3400[301]; s.c. 300[313]
Order: Anura	Phenylethalymine bases: Dopamine, noradrenaline, adrenaline[308–310]. Tryptamine derivatives, at least 10 compounds were identified and isolated; one of the most common is bufotenine (N-N-dimethyl-5-hydroxy-tryptamine) (Figure 1[308,311,312]). Histamine and its derivatives[308,313,314]. Peptides such as bradykinin[315], physalaemin[316] and others[313,317,318]. Proteins, labile highly hemolytic, toxic to mammals, m.w. 35,000[319]. Alkaloids: bufogenins (C_{24}-steroids), about 20 different compounds were isolated[320]. Bufotoxins, conjugates of bufogenins with syberylarginine[320]. M) Batrachotoxin (see Figure 1), highly potent[321,322,313]. Pumiliotoxin (Figure 1[313]). Histrionicotoxin, acetylenic and allenic alkaloids with a spiral ring system[324]. N) Atelopidtoxin[325], structure not defined.	M) s.c. 2[313]; s.c. 1500[313] N) i.p. 16[325]
Class Reptilia Order: Sauria genus: *Heloderma*	Toxic-lethal protein(s). Kinin releasing and arginine esterolytic factor (m.w. 76,000). Arginine – esterase (m.w. 25,000), hemorrhagic and lethal[326,327]. Other enzymes: L-amino oxidase, PhA, hyaluronidase, fibrinolytic, trypsin like[328].	
Order: Ophidia (snakes)	Low molecular single chained basic proteins: a) 61–62 amino acids 4, disulfide bridges (elepidae[106–117], hydrophiidae[101–105]), b) 71–74 amino acids, 5-disulfide bridges (elapidae[104,105,109,110]). c) Cardiotoxin group, strongly basic 57–62 amino acids, 4 disulfide brigdes including: the cardiotoxin[111–113], cobramine[111], cyto-	(m) C.v., i.p. 440 (m) Txn, i.p. 74 (m) Cxn, i.p. 1480 (m) PhA, i.p. 5000[11]

Table I (continued)

Animal group	Venom composition	Mice LD_{50} (μg/kg)
	toxin[329], toxin γ[330], DLF[331]. Other neurotoxins: β-bungarotoxin: 179 amino acids, m.w. 28,500[41,116]. Viperotoxins: *V. palestinae*, 108 amino acids, 3 disulfide bridges, m.w. of 12,333[332]. *V. ammodytes*, 145 amino acids, m.w. 16,153[333]. Crotalotoxins from the venom of *Crotalus durissus terrificus*: Crotamin: 46 amino acids, m.w. 5474, pHi 10.3[334,335]. Crotoxin: m.w. 21,000 – composed of an acidic component of 76 amino acids and a basic protein of 110 amino acids[334,335]. Convulxin, an acidic protein (pHi 6)[338,41]. Enzymes: Phospholipase A[19,28,339–341]. Endopeptidases, trypsin like[342–344]. Exopeptidases (amino)[345–346]. Arginine ester hydrolases, in crotalid and viperid venoms partly responsible for their bradykinin releasing and coagulant activities[90,347–349]. Hemorrhagins devoid of proteolytic activity[90,63]. Phosphatases[90] – phosphodiesterase[46,203]. DNAse[59,125]. RNAse, AMPase[240]. ATPase[253,252], phosphomonoesterases[59,65], phospholipase c[90,329]. L-amino oxidase[43,138]. Hyaluronidase[47,48,350], Acetylcholinesterase[181,217]. Biogenic amines: Acetylcholine, tryptamine derivatives, (such as 5-HT, bufotenine)[90,119,239].	
Class Mammalia Order: Monotremata	Constituents not identified[220].	
Order: Insectivora	Toxic principle probably a protein[224].	

Abbreviations: i.v., intra venous; s.c., subcutaneous; i.p., intraperitoneal; p.o., Per oss; 5-HT, 5-hydroxytryptamine; PhA, phopholipase A; C.V., crude venom; Txn, Toxin; Cxn, cardiotoxin. a) from the honey bee; b) Melittin; c) Apamin; d) MCD-peptide; e) PhA; f) from the scorpion *A. australis*; g) toxin II of *A. australis*; h) from the scorpion *L. quinquestriatus*; i) toxin V of *L. quinquestriatus*; j) from *Latrodectus mactans tredecimguttatus*; k) from *Synanceya*; l) partly purified material from *Scorpaena*; m) from *Naja naja atra* and the purified compounds are all derived from this venom.

mixtures. The pathology of venoms is generally a consequence of the combined effects and interactions of the different constituents.

Interactions

The interaction between venom constituents may be expressed in a direct form resulting in the formation of a new toxic product. This has been demonstrated in repulsive mechanisms of certain arthropods.

The pygidial defensive gland system in the bombardier-beetle (*Brachynus*) is composed of several compartments; in the collecting bladder, hydroquinone and hydrogen peroxyde solutions are accumulated and then transferred into chitin capsule (explosion chamber). Unicellular glands, connected to this chamber, excrete their contents containing the enzymes catalase and peroxidase. Catalase decomposes hydrogen peroxide into water and oxygen, and peroxidase oxidizes the hydroquinone. The consequence of this interaction is an audible explosion with the release of a hot mixture composed of oxygen (the source of the pressure), water, benzoquinones, hydroquinones and hydrogen peroxide[13–15]. The unusual feature of *Brachynus* catalase, to act optimally at high temperature of 70°–80 °C, may represent a special adaptation[16]. Principally the same mode of interaction was found in the repulsive secretion of the millipede *Apheloria*. The excreting glands consist of two compartments, one storing a cyanogenic compound in the form of mandelonitrile, and the second an enzymatic factor that catalazes its hydrolysis. When the contents of the two compartments are mixed, mandelonitrile is converted into benzaldehyde and hydrocyanic acid which is released into the atmosphere[17]. Both, in the case of the beetle and millipede, stable and non-toxic precursors are stored and accumulated while the toxic compound is produced on the spot upon need. The elegance and the efficiency of these mechanisms follows from the suitability of the specific anatomical arrangements to the chemical reactions.

Another form of interaction between venom constituents results in a synergistic effect due to a simultaneous action on the same target tissue. This has been demonstrated in direct lysis of erythrocytes due to the synergic interaction between phospholipase A (PhA) and the direct lytic factor (DLF) in snake and bee venoms[18]. The enzyme PhA, which is widely distributed in animal venoms (Table I and [19–29]), catalyzes the hydrolysis of phosphatides at the ester bond adjacent to the phosphoryl alcohol linkage releasing a fatty acid and the hemolytic agent lysolecithin[30–33]. Purified PhA is unable to lyse washed erythrocytes due to the lack of an external phosphatide source and its inability to split the membrane-bound phospholipids of the intact cell[34–36]. However, the simultaneous presence of the enzyme and DLF results in strong hemolysis due to the conversion of the cell's membrane-bound phospholipids to lysophosphatides[35–38]. Thus, the function of DLF may be considered as limited to the facilitation of the interaction between

PhA and the membrane-bound phospholipids which cannot be hydrolyzed by the PhA alone. It is suggested that the DLF, a strongly basic polypeptide, may act through the formation of electrostatic bonds between its cationic charges and the acidic groups in the erythrocyte membrane thus binding the PhA with the membrane phospholipids[35,37–39]. The potentiation of DLF's axonal conduction blocking ability[40,41] cardiotoxicity and hemmorhaging ability[11,42] by phospholipase A may be considered as additional manifestations of the interaction between these two constituents[90].

An additional form of cooperative action is expressed in the facilitation of penetrability of a toxic component(s) by another factor nontoxic by itself. The simplest manifestations of this mode may be seen in mixtures of repulsive secretions of certain arthropods used in nature against predatory insects. The toxic agent is a concentrated carboxylic acid which is accompanied by lipophilic factors enabling its penetration through the wax layer of the insects epicuticle. In many beetles the repulsive agent is the cytotoxic formic acid, accompanied by lipophilic aliphatic hydrocarbons such as n-undecane and n-tridecane[43,16]. Equally the defensive spray of the whip scorpion *Mastigoproctus* consists of acetic acid (84%), the primary irritant, water (11%) and caprylic acid (5%) – which serves as a wax solvent thus enabling the penetration and spreading of the acid[44,17].

Neurotoxic proteins (and as such limited in their penetrability) make up the main lethal factor in many venoms as those of elapid and hydrophiid snakes, scorpions, spiders, venomous fishes and cnidarians.

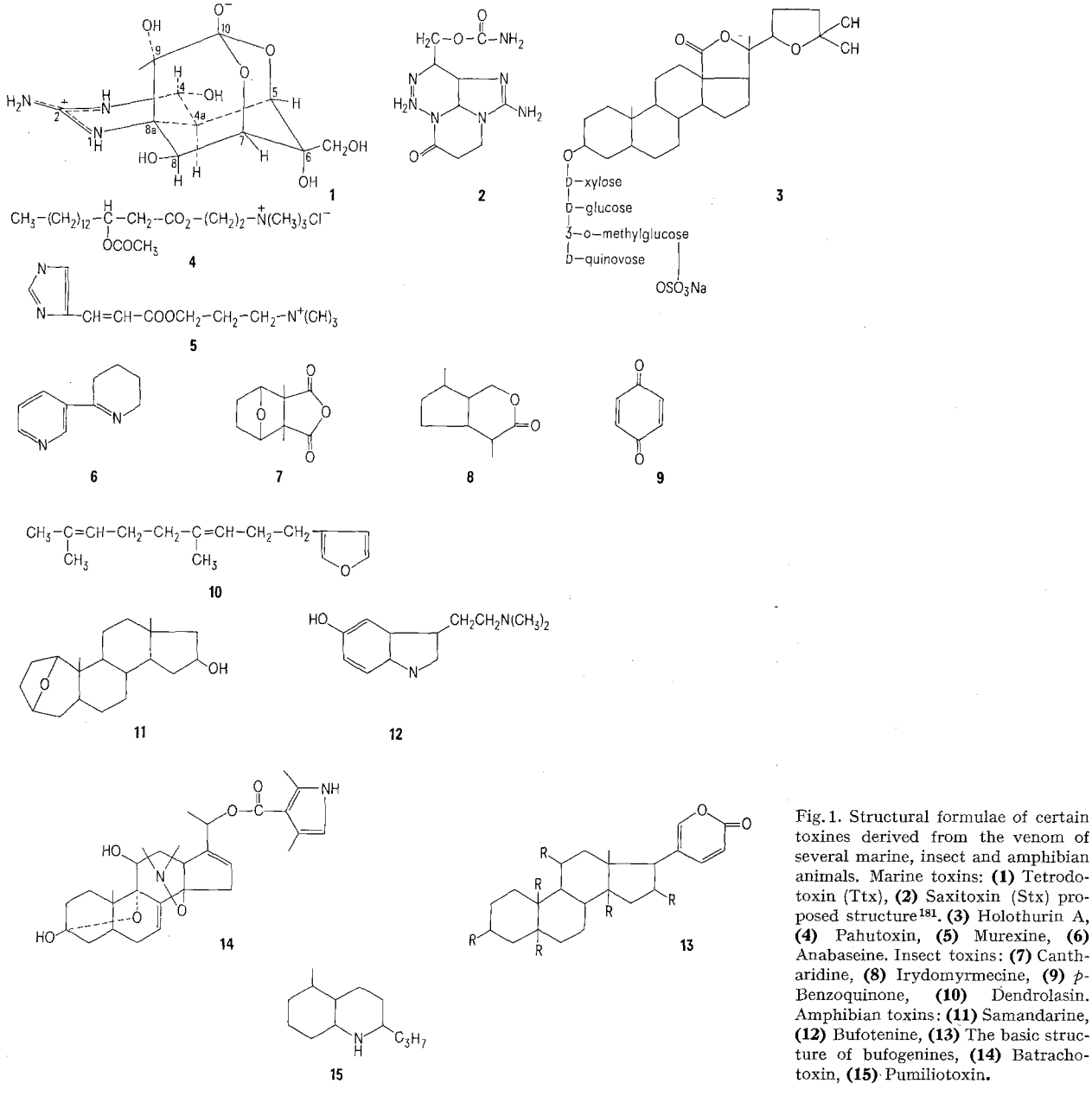

Fig. 1. Structural formulae of certain toxines derived from the venom of several marine, insect and amphibian animals. Marine toxins: **(1)** Tetrodotoxin (Ttx), **(2)** Saxitoxin (Stx) proposed structure[181]. **(3)** Holothurin A, **(4)** Pahutoxin, **(5)** Murexine, **(6)** Anabaseine. Insect toxins: **(7)** Cantharidine, **(8)** Irydomyrmecine, **(9)** *p*-Benzoquinone, **(10)** Dendrolasin. Amphibian toxins: **(11)** Samandarine, **(12)** Bufotenine, **(13)** The basic structure of bufogenines, **(14)** Batrachotoxin, **(15)** Pumiliotoxin.

Generally, (as it may be evident from Table I) the neurotoxic factors are accompanied by the presence of different enzymatic constituents supposed to contribute to their penetrability. This quality is mainly attributed[46] to hyaluronidase, whose presence was demonstrated in snake[47,48], arthropod[46,48–51], cnidarian and echinodermal[46] venoms. Hyaluronidase is identified with the spreading and diffusing factors of venoms[46]. However, it should be noted that hyaluronidase caused only few observable changes in the structure of the sheath of a giant axon and did not increase its permeability[52,53]. Equally, phosphodiesterase, L-amino oxidase, proteolytic enzymes, a direct lytic factor from ringhals venom and a neurotoxic fraction from cobra venom did not influence the permeability in squid axons, and were unable to render them sensitive to curase[54–57]. On the other hand, these actions could have been performed by PhA[52,58]. It has been found that an isolated fraction of PhA increased permeability and sensitized axons to curare and acetylcholine[40] and mimicked the action of crude snake venom in blocking neural conduction, production of vesiculations of the Schwann cells and destruction of the permeability barrier to the penetration of radioactive acetylcholine. These effects of PhA are attributed to the formation of lysophosphatides[52]. The effects of PhA on axons could have been mimicked by a lipid extract of venom-axon tissue incubate, as well as by pure lysolecithin[52,59,60].

In spite of the general importance of protein neurotoxic factors, the combined action of enzymes plays an essential role in the pathology of crotalid and viperid snake venoms[61]. The myonecrotic effects are generally attributed to proteases[10,62–64]. However, myolysis may also be obtained as a result of a synergistic interaction between PhA and certain non-enzymatic proteins[27]. Although the action of certain hemorrhagins in these venoms appears to be independent or devoid of proteolytic activity[10,11,65,66], certainly their effect is increased by the action of proteolytic enzymes and anticoagulation agents[67,68].

In this connection it should be noted that synergistic interactions were demonstrated between different neurotoxins derived from scorpion venoms[69,70].

Neurotoxins

It appears that difficulties concerning the criteria for definition of the term toxin[71,72] may equally be applied to the term neurotoxin. The chemist tends to consider as neurotoxin any potent lethal factor devoid of enzymatic activity. The physiologist would accept as neurotoxin a substance which interferes with the normal response of a neurophysiological preparation. Actually, the definition of a neurotoxin demands experimental confirmation of a specific affinity of the compound to an excitable tissue. Several such compounds derived from the venom of certain animals are presented here.

Tetrodotoxin (Ttx) and Saxitoxin (Stx) (Figure 1) are unique in their high toxic potency (Table I) and their specific action. The block of the normal rising phase of the action potential of an excitable tissue is considered as the primary effect of these toxins. As non-depolarizing blockers, Ttx and Stx were used as pharmacological tools in the study of phenomena such as ionic conductance and synaptic transmission[73]. It has been concluded[43,74–78] that those materials selectively depress inward membrane permeability to sodium ions. This quality is attributed to their cationic nature which enables their binding to negatively charged sites within the membrane and thus blocking the passage of sodium ions[79,80]. Their precise mode of action at the molecular level still remains unknown. However knowledge of the structural configuration of tetrodotoxin (Figure 1)[81,82] enabled certain reasonable postulations concerning its mode of action. Attention was mainly directed to the guanidinium group as the receptor binding site. However, a shift in the pH from 7 to 9, where the guanidinium group still remains strongly positively charged, resulted in an apparent loss of activity, suggesting the importance of the hemilactallactone part of the molecule in maintaining the biological activity[83–85]. Furthermore, different compounds including Ttx derivatives all possessing the guanidinium group demonstrated a low activity[82,86,87]. At present it is considered that Ttx interacts with a receptor both by an electrostatic link of the guanidinium group and through a hydrogen bonding at the negatively charged oxygen at C_{10}[83,88]. This is supported by the fact that the rate of activity of a series of Ttx derivatives correlates with the rate of negativity of the C_{10} oxygen[80].

According to Table I, it is evident that the essential constituents of venoms which are introduced through stinging or biting devices are non-enzymatic highly toxic proteins.

The low molecular basic proteins derived from elapid and hydrophiid snake venoms are the most extensively studied and have been recently presented in a series of excellent reviews[12,41,89–91]. Thus, we shall discuss only certain essential data concerning their structure.

From a functional point of view, the above toxins may be divided into 3 groups: 1. Low molecular weight basic neurotoxic proteins performing a neuromuscular block of the non-depolarizing type in vertebrate systems. This is due to a competitive block of the cholinergic receptors at the post-synaptic membrane of the junction[28,92,94–97]. In this group are included two types of toxins: Type 1, present in hydrophiid[98–100] as well as elapid[101–105] venoms. They are composed of a single chain of 61–62 amino acids cross linked by 4

disulfide bridges of an identical location[103,106–109] and of a molecular weight of 6,700–7,000 (see Figure 2). Type 2, toxins including the α-bungarotoxin, are single chained proteins of 71–74 amino acids cross linked by 5 disulfid bridges with a mol. wt. of about 7,800[104,105,109,110]. This group of toxins makes up the main lethal factor of the above snake venoms.

2. The cardiotoxin group – these are strongly basic (pH above 12) polypeptides, the most abundant constituents in elapid venoms (22–25% of the dry weight[111–113]). They are composed of a single polypeptide chain of 57–62 amino acids cross linked by 4 disulfide bridges with a molecular weight of about 6,000–7,000[111–113]. These toxins have a low lethal potency which is about 1/20 that of the first neurotoxic group and 1/3 that of crude venoms[41]. However, they may perform a wide range of pharmacological actions such as constricture of skeletal[91,114], smooth[91] and heart muscle[91], blockage of axonal conduction and ganglionic transmission[141], a direct lytic action and a synergistic effect with PhA (see previous chapter). The comparative analyses of the primary structures of type 1 and type 2 neurotoxins, as well as those of the cardiotoxin group[92,41], demonstrate a clear resemblance and enable speculation on the possible phylogenetic relations among them[91].

3. This group is, up to now, represented only by β-bungarotoxin which markedly differs from the others discussed in its chemistry as well as mode of action. This neurotoxic protein is composed of 179 amino acids with a molecular weight of 28,500[41,116] and it blocks the neuromuscular junction due to a presynaptic excitatory effect resulting in depletion of terminal vesicles and anatomical damage[41,117,118].

From the structure-functional aspect the first group of toxins, which makes up the main lethal factor of elapid and hydrophiid venoms, has received the greatest attention. A comparison of the full primary sequences of eight hydrophiid and elapid toxins demonstrates an identity in the location of 22 amino acid residues[41] – besides the disulfide bonds, as indicated in Figure 2. Thus it might be suspected that these residues play an essential role in the biological activity. Cobrotoxin derived from the venom of *Naja naja atra*[107] presented in Figure 2, was exposed to a series of chemical modifications. Reduction of the disulfide bonds results in a complete loss of activity[121]. Upon reoxidation full toxicity was regained[121] as well as the ORD and CD patterns of the native toxin, indicating a β-structure[120,121]. It has been found that the integrity of the following functional groups in the molecule is essential for the maintenance of its toxic activity: Lysine in Step 47[122], tyrosine in step 25 (which exists originally in a 'buried' position)[123], the sole tryptophan in position 29[124] and the masked carboxyl group in Glu 21[122,126]. It should be noted that the removal of a tetrapeptide from the C-terminal end of certain neurotoxin from *N. naja siamensis*[127] resulted in the loss of only half of its toxicity.

This group of toxins have recently been employed as tools in the study and isolation of the cholinergic receptor in vertebrate neuromuscular systems. *Naja nigricollis* α-toxin coupled with sepharose granules served as a device for a selective absorption of a cholinergic receptor protein[128]. The use of I^{125}-α-bungarotoxin enabled[129] the estimation of the number (4.0×10^7) of acetylcholine receptors per motor endplate in a rat diaphragm as well as the isolation of a phospholipoprotein from the electric tissue of *Electrophorus*[130,131].

Scorpion venoms serve as an additional source of low molecular neurotoxic proteins[132–137,69,139,142]. It may be supposed that the depolarizing and blocking effects of the crude scorpion venoms on nerves[141,142], muscles[143,144] and the neuromuscular junctions[145,146] in vertebrate systems are due to these toxins. Recently, 15 toxins derived from several scorpion venoms[147,148] have been isolated and purified. The amino acid composition of these toxins is presented in Table II. It has been found[147,149] that they are single chained mainly basic polypeptides cross linked by 4 disulfide

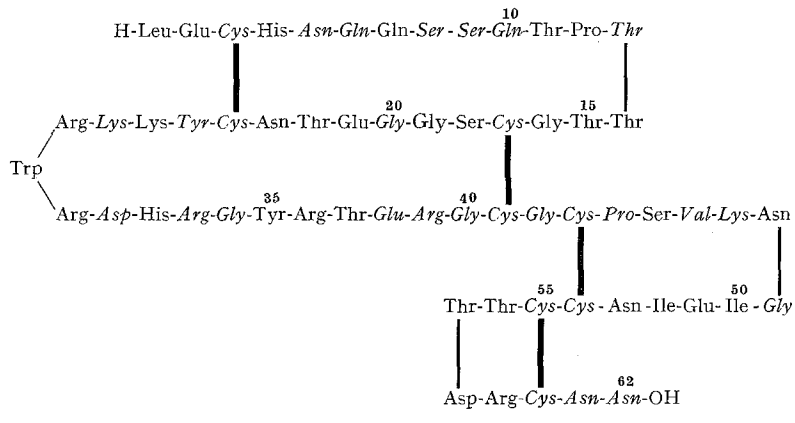

Fig. 2. The structure of cobrotoxin (YANG 1970[107]). The underlined amino acids were found to be identical in all toxins belonging to this type derived from elapid as well as hydrophiid snake venoms.

bridges. Examination of Table II reveals that, at the level of amino acid composition, these compounds have some features in common: Low molecular weight (varying between 6,500 and 9,000 with the majority around 7,000); absence of methionine (a common feature of the majority of snake toxins as well); presence of 8 half cystines (except in one toxin) which according to MIRANDA et al.[147] all are involved in disulfide bridging; low histidine and phenylalanine contents. Beyond these resemblances in their composition they appear to be quite diverse and are much more heterogenic than the elapid and hydrophiid snake toxins[91,92]. Considering the heterogeneity in composition, it is interesting to notice the relatively high level of homogeneity in their n-terminal primary structure. This is demonstrated in Figure 3 presenting the n-terminal amino acid sequences of 6 toxins[142] and the full sequence of 2 toxins[150,151] grouped and arranged in a form so as to provide maximal homology. Comparing the full primary structure of toxins AI, I' to that of toxin AII (Figure 3[140,151]) it is evident that the greatest extent of homology appears at the n-terminal section of the molecule which gradually decreases towards the C-terminal end. Thus, it has been suggested[149] that the n-terminal sequence might play an important role in the biological function. Furthermore, considering the rate of activity of the above toxins (Figure 3), it has been proposed that the amino acid in position 10 is of particular importance and the replacement of a neutral residue (as in the highly active AII) by a basic residue might be associated with a decrease of the specific toxicity[149].

Often the composition and chemistry of venoms can be used as a tool in animal taxonomy and phylogenesis. The classification of toads from the genus *Buffo*[152], the differentiation between species of snakes from genus *Bothrops*[153], *Laticauda*[154], subspecies of *N. haja*[155] and *L. mactans* spiders[156] may serve as examples. The structure of the toxins derived from the venom of the scorpion *Androctonus australis* Hector may serve as a typical example of taxonomical implications derived from chemical information. It has been found that venom collected from scorpions originating from two different geographical regions in North Africa contain different toxins. The venom from Tozeur (Tunisia) scorpions contains toxin AI, AII and AI' and that

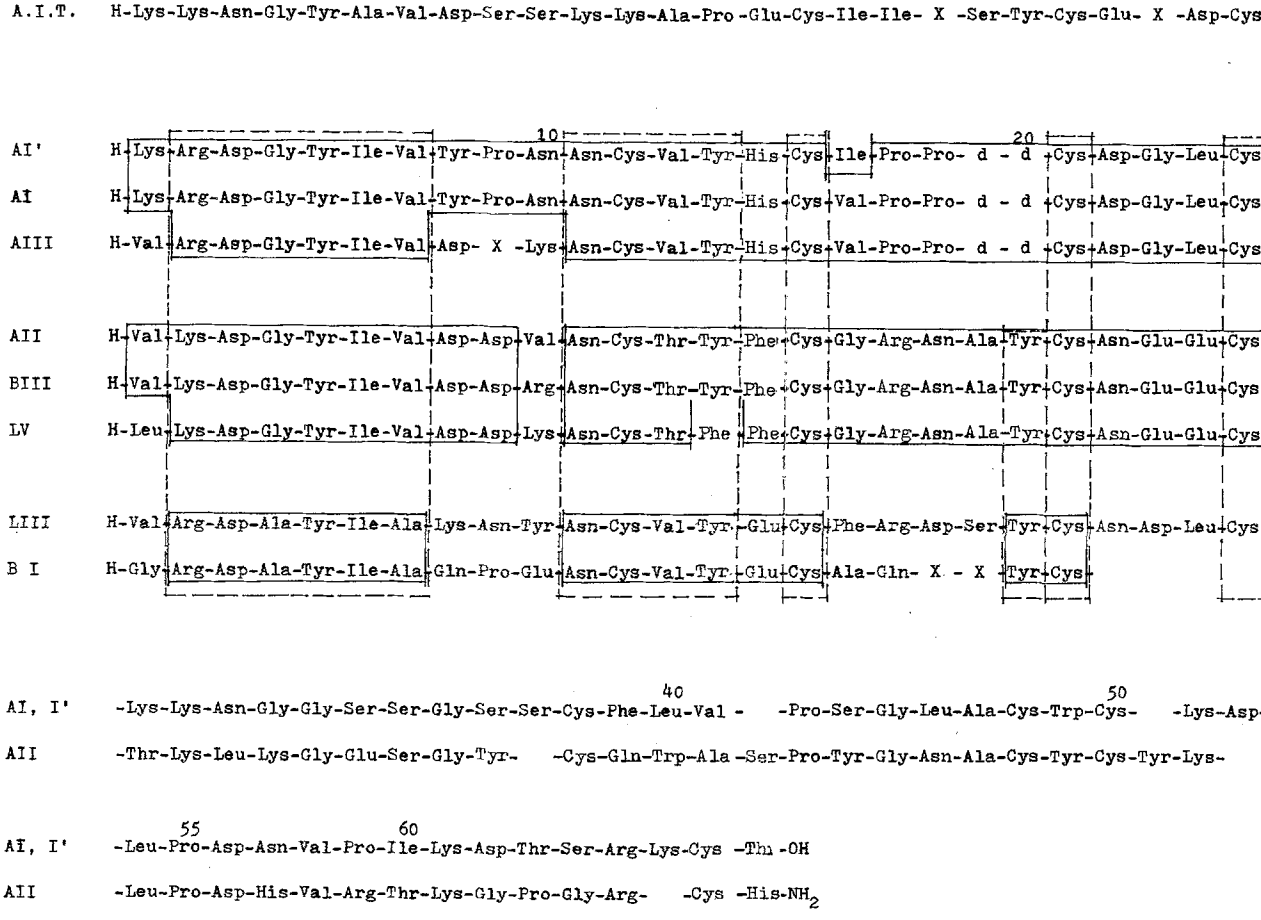

Fig. 3. Amino acid sequences of toxins derived from scorpion venoms. The toxins are indicated by roman numbers and their origin is indicated by the letters A, B and L corresponding to the venom of *A. australis* *B. O. tunetanus* and *L. quinquestriatus* respectively (MIRANDA et al. 1970[147], ROCHAT et al. 1970[149], 1971[150], 1972[151]). AIT indicates the insect toxin derived from the venom of *A. australis* (ZLOTKIN et al. 1971[171]). X, not detected; d, deletion.

Table II. Amino acid composition of scorpion toxins

Amino acid	*C. sculpturatus*[a]				*L. quinquestriatus*[b]					*B. o. tunetanus*[b]			*A. australis*[b]				
	TxI	TxII	TxIII	TxIV	TxI	TxII	TxIII	TxIV	TxV	TxI	TxII	TxIII	TxI	TxII	TxIII	IT[c]	C.T.[d]
Aspartic ac.	7	5	7	7	10	10	9	9	10	9	9	9	9	8	8	11	6
Threonine	5	5	4	1	1	2	1	3	1	2	1	3	2	3	0	4	4
Serine	2	6	2	4	2	4	3	3	3	2	2	2	6	2	6	6	4
Glutamic ac.	6	5	8	9	2	2	2	3	4	5	5	4	0	4	0	3	10
Proline	4	3	3	2	3	4	2	3	2	4	3	3	6	3	6	1	2
Glycine	10	9	9	9	8	5	5	7	7	5–6	6	7	6	7	6	4	4
Alanine	0	0	3	4	4	2	5–6	4	3	4–5	5	3	1	3	3	3	2
Half-cystine	8	6	8	8	8	8	8	8	8	8	8	8	8	8	8	8	10
Valine	1	1	3	3	5	3	3–4	3	2	2	2	4	5	4	6	3	4
Methionine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Isoleucine	1	1	0	0	4	1	2	4	2	3	4	1	2	1	3	2–3	2
Leucine	5	3	6	8	3	2–3	2	3	2	3	3	1	4	2	4	5–6	2
Tyrosine	6	4	8	7	3	5	6	5	6	4	5	7	3	7	3	5	4
Phenylalanine	2	2	3	1	1	0–1	1	0	2	1	1	1	1	1	1	1	0
Lysine	9	10	8	9	4	4	4	6	8	4	5	5	6	5	6	7	4
Histidine	1	1	1	1	3	1	1	0	0	1	1	1	1	2	2	1	0
Arginine	1	1	2	2	1	2	3	3	3	2	3	4	2	3	1	1	9
Tryptophan	2	2	3	3	2	1	2	2	2	2	2	1	1	1	1	1	2
Total	70	64	78	78	64	57	60	66	65	62	65	64	63	64	64	67	69
Mol. wt.	7900	7108	9002	8873	6928	6511–6545	6764–6792	7313	7462	6919–6933	7539	7270	6808	7249	6826	7498	8104

[a] McIntosh and Watt 1972[148]; [b] Miranda et al. 1970[147]; [c] Zlotkin et al. 1971[171], the insect toxin.; [d] Zlotkin et al., unpublished[173], the crustacean toxin.

from Chellala (Algeria) contains toxins AI, AII and AIII (see Figure 3). Toxins AI' and AIII may be considered as isotoxins to AI, the first differing only by a conservative amino acid change in position 17 and the second by greater number of amino acid residues (Figure 3). Up to now the subspecies *Androctonus australis* Hector has been considered, from the taxonomical point of view, as being homogenous[157]. However, the interchange between toxins AIII and AI', which must be a genetic characteristic, suggests a taxonomical heterogeneity. Recently, the taxonomy of *A. australis* has been reconsidered and constant morphological differences between scorpions of both geographical origins were found[147]. In this connection it should be noted that recently the amino acid sequences of 11 elapid and hydrophiid venoms were used in the construction of a phylogenetic relationship between these toxins[158].

Selective toxins

From an ecological point of view, the use of chemical means by organisms, for purposes of defense or obtaining food, may be considered as an efficient and energy sparing solution. Thus the wide distribution of poisonous mechanisms in the animal kingdom (Table I), is an expression of their high survival value.

In this connection one would expect to find a certain relation between venom composition and activity to the organisms which constitute the natural environment of the venomous animal, either as enemies or as prey.

In certain instances such relations could have been noticed as expressed in the selectivity in the action of venoms. This has been demonstrated in the venoms of parasitic wasps affecting a single or few species of insects only[159,160] or in the venoms of conidae[161,162] which are classified according their specific toxicity as piscivorous, molluscivorous and vermivorous. Equally, it has been found that the specific toxicity of a substance derived from the pedicellariae and spines of certain sea urchins[163] or the tentacles of a sea anemone[164] to crustaceans is due to two labile protein factors and to a low molecular basic protein, respectively.

However the majority of venoms like those of reptiles[165], scorpions[166] and spiders[167] have been found to contain lethal potency not only to mammals and arthropods but even to unicellular organisms[168]. Relating venoms' composition to their biological activity, it may be assumed that the lethality of different organisms is due either to the same factor(s) in the venom (affecting a certain essential tissue or physiological process common to all organisms) or to diverse factors affecting selectively different groups of organisms. Accumulating experimental evidence points to the validity of the latter assumption.

With the aid of column electrophoresis and ion exchange chromatography, the venom of the black widow spider was separated into several protein fractions (m.w. about 50,000) differentially active on insects and mammals[167]. One of the fractions exhibited a fast knock-down effect on flies, a second exhibited a slow toxic effect on insects, while the third one was shown to be toxic to mammals and inactive to insects[167].

This phenomenon of selective toxicity was recently emphasized by the study of scorpion venoms. It has been shown[168] that when the venoms of 18 different species of scorpions were tested for their lethality to mice and paralysis of insect larvae, no correlation was found between the two biological activities. Furthermore, purified toxins highly active to mammals, from the venom of *A. australis* ([157] – see previous chapter) were inactive when applied to different insect species as well as other arthropods[170,166]. By the aid of a specific biological test based on the contraction paralysis response of blowfly larvae[169], and the employment of gel filtration and ion exchange column chromatography, a toxic protein (the insect toxin) was purified from the venom of the scorpion *A. australis*. The final product, with a 267-fold purification and a toxicity yield of 95%, is a single chained protein composed of 67 residues (see Table II) with a molecular weight of 7,498 cross linked by 4 disulfide bridges. The n-terminal sequence is presented in Figure 3 and the c-terminal was established as Thr-Ile-OH[171]. It is interesting to note that the comparison of the primary structure of the present toxin with that of 8 mammal toxins (Figure 3) reveals a similarity in the sequence of the first 7 positions and the presence of half cystines at positions 16,22 and 26, thus suggesting the location of disulfide bridges. However all other positions are occupied by different amino acids. The presence of a disulfide bridge in position 12 is common to all scorpion toxins active on mammals (Figure 3), whereas it is occupied by lysine in the insect toxin. At this stage we cannot make any deductions concerning the structure function relationships of the insect toxin unless additional structural information will be obtained. The presence of discrete proteins specifically active on mice and fly larvae was demonstrated in six additional scorpion venoms[172].

In contrast to the crude venom of *A. australis* scorpion, the insect toxin was inactive when injected into an arachnid and a crustacean[166]. With the aid of column chromatography, using the lethality of isopod terrestrial crustaceans as a biological criterion, an additional selective toxin specifically toxic to crustacea was isolated and purified([166,173] and Table II). Both the insect toxin and the crustacean toxin may be considered as neurotoxins. The former in contrast to mammal toxins was able to block the induced afferent transynaptic response at the sixth abdominal ganglion of the crockroach[174]. The crustacean toxin, in contrast

to insect and mammal toxins, was able to induce an excitatory block of the crayfish stretch receptor organ[175].

Most recently it has been found[165] that in contrast to the high potency of elapid crude venoms to fly larvae and isopods, their toxins (isolated and purified according to the criterion of mice lethality) are completely inactive[165]. The venom of *Naja mocabique* was separated by gel filtration and it was found that the lethality to arthropods was due to proteins completely different from those lethal to mice[165]. Thus it may be concluded that the presence of selective protein toxins specifically active to arthropods is not only limited to arachnid venoms but is equally shared by snake venoms.

It should be noted that scorpions as predators feed mainly on insects. Thus, from an ecological point of view the insect toxin should be considered as the essential component of the venom. On the other hand, according to paleontological findings, the ancient water scorpions-eurypterides lived in close association with vertebrates – the ostracodermic and placodermic fish[176]. Possibly, the phenomenon of selective toxins may represent a chemical adaptation to changes in food sources during evolution.

Whatever may be the speculation on the ecological significance of these toxins, it is evident that due to their selectivity they may serve as valuable tools in the study of comparative physiology and neurochemistry.

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