Chemistry of Animal Venoms, Poisons and Toxins

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Toxic products from animals have attracted steadily increasing interest during the last years. New methods and techniques for isolation, purification and structural analysis of these natural compounds enabled a rapid progress in our knowledge of their chemistry and mode of action. Various toxins and components from venoms and poisons were applied in biochemistry, pharmacology and medicine as valuable tools.

This article can give only a short summary of the chemistry of animal poisons, venoms and toxins. For further reading, the proceedings of the 3 major international symposia[1-3] and the various review articles mentioned in each chapter are recommended.

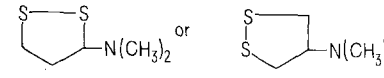
A) *Protozoa*

Paralytic shellfish poisoning and mass mortalities of marine animals were found to be caused by several species of marine dinoflagellates of the genera *Gonyaulax, Gymnodinium, Prorocentrum* and *Prymnesium*. These organisms produce powerful toxins and hemolytic substances which accumulate in the hepatopancreas or the siphons of shellfishes which filter plankton from the water. Thus, 'saxitoxin', the compound held responsible for the toxicity of shellfish, was so named because of its close association with the Alaskan butter clam *Saxidomus giganteus*. Toxins isolated from clam and mussel tissue were found to be identical with the paralytic toxins from *Gonyaulax catenella*[4,5]. Experiments revealing the same pharmacological effects support the assumption of the identity of both substances[6]. The purified toxins seem to be a heatstable derivative of a purine base[7], the complete chemical structure has not yet been established.

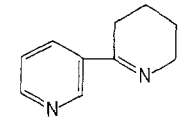
B) *Metazoa*

1. *Coelenterata.* From alcoholic extracts of sea anemone tentacles, tetramethylammonium hydroxide ('tetramine') has been identified as the major pharmacologically active component[8], amounting to about 1 to 2 mg/g tissue in *Anemonia sulcata* and *Actinia equina*[9]. Other toxins isolated from nematocysts of cnidarians seem to be of protein nature. LARSEN and LANE[10] found that the toxin of *Physalia physalis* is a thermolabile, nondialyzable protein which exhibits in warm-blooded animals remarkable toxicity. From tentacle extracts of the Australian sea wasp or box jellyfish *Chironex fleckeri*, CRONE and KEEN[11] isolated two proteins showing both lethal activity; moreover, one of them possessed strong hemolytic properties. The molecular weight of the hemolysin and the other toxic component has been estimated to be 70,000 and 150,000, respectively. A toxin obtained from the nematocysts of *Actinia equina* had a molecular weight of 13,000; amino acid analysis showed a relatively high proportion of basic amino acids as well as of aspartic and glutamic acid, but no cysteine was present[12].

2. *Vermes.* Carnivorous insects die usually from feeding on the dead body of the marine annelid *Lumbriconereis heteropoda*. NITTA[13] isolated and crystallized the toxic principle from the worm, HASHIMOTO and OKAICHI[14] described the structure of 'nereistoxin' to be a tertiary amine with a cyclic disulfide similar to α-lipoic acid. A toxin from the hoplonemertine worm, *Paranemertes peregrina*, has been identified as



Nereistoxin



Anabasein

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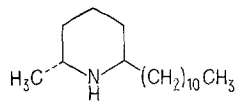
anabaseine (2-(3-pyridyl)-3,4,5,6-tetrahydropyridine) which is the first report on the occurrence of this compound as a natural product[15]. It is the only paralyzing constituent of the proboscis venom protecting the animal against potential predators.

3. *Arthropoda. Insects.* Insects produce a lot of secretions consisting of chemically different substances: those of relatively low molecular weight like acids, aldehydes, ketones, esters, hydrocarbons, lactons, phenols and *p*-benzoquinones, or others like peptides or proteins of apparent higher molecular weight. They use them as attractants for chemical communication or as repellents exhibiting malodorous or distasteful properties. Although a general classification of a substance as attractant, repellent, venom or poison seems often difficult, this review will concentrate only on insect venoms which, in their primary function, are used to subjugate a prey. However, this does not exclude the fact that they have also defensive functions. For further information on defensive secretions of insects see reviews of EISNER and MEINWALD[16], WEATHERSTON[17], PAVAN and DAZZINI[18].

Bees and wasps produce venoms which contain, besides histamine and other biogenic amines, polypeptides of various pharmacological properties and few enzymes: phospholipase A and B and hyaluronidase[19]. From wasp venoms (*Vespa vulgaris, V. polistes*) peptides with kinin-like activity contracting smooth muscle have been isolated and their amino acid sequence elucidated[20,21]. In bee venom (*Apis mellifica*) 3 toxic polypeptides have been detected: the hemolytic melittin, the neurotoxic apamin and a mast cell degranulating (MCD) peptide. Melittin shows in its primary structure a rather characteristic arrangement of basic hydrophilic residues at the carboxyterminal part (position 21–26), and of hydrophobic residues at the aminoterminal part of the molecule (position 1–20) which accounts for its strong surface activity[22]. Apamin, consisting of 18 amino acid residues, is the smallest neurotoxic polypeptide known[23].

Venoms of ants consist either of proteinaceous or alkaloidal components. Hyaluronidase and a heat-labile hemolytic protein have been found in the venom of the Australian bull ant, *Myrmecia gulosa*[24]. Ants of the genera *Solenopsis* and *Atta* are the only hymenopterans known to produce alkaloids in their venom glands. BRAND et al.[25] isolated from the venom of *Solenopsis geminata, S. xyloni* and *S. saevissima* various alkaloids of the 2,6-disubstituted piperidine type called 'solenopsin'[26].



Solenopsin A

Spiders. Free amino acids, peptides and proteins were identified as the main constituents of spider venoms. A toxin of polypeptide nature with a molecular weight of about 5,000 has been separated from *Latrodectus mactans* venom[27] and of about 7,000 from *Pterinochilus spec.*[28] and *Dugesiella hentzi* venom[29]. Moreover, marked hyaluronidase and proteolytic activity was detected in Brazilian spider venoms[30,31], phosphodiesterase activity in the venoms of *Atrax robustus, Latrodectus mactans* and *Aphonopelma cratuis*[32].

Scorpions. Venoms of scorpions contain several toxic, mostly basic polypeptides. From *Androctonus australis, Buthus occitanus tunetanus,* and *Leiurus quinquestriatus quinquestriatus* 11 neurotoxins were purified. All possess a molecular weight of about 7,000 consisting of a single peptide chain with 57–66 amino acid residues cross-linked by 4 disulfide bridges[33]. The amino acid sequence of 6 of them, elucidated until the 22 to 26 residue from the amino terminus, reveals a high degree of homology, suggesting that this part of the molecule may play a major role in the biological activity of the toxins[34]. The complete sequence of toxin I and II from *Androctonus australis* shows a homology of nearly 50%[35,36].

4. *Mollusca.* In the extracts of hypobranchial glands of several gastropods, cholinesters have been identified as the main pharmacologically active substances: 'murexine' (β-imidazolyl-acryloylcholine)

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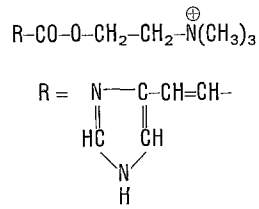
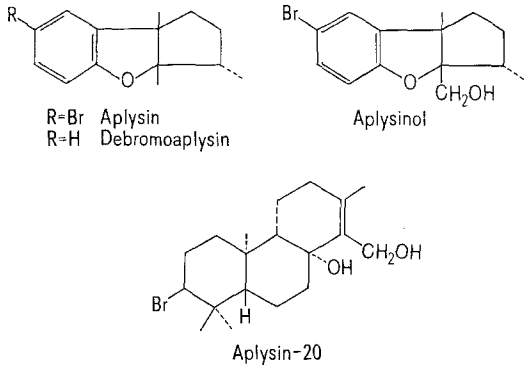
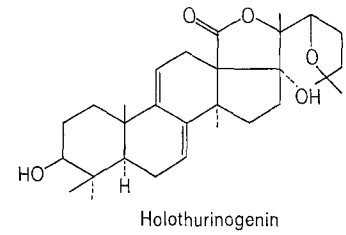
from *Murex* species[37], seneciolylcholine (dimethylacryloylcholine) from *Thais floridiana*[38], and acrylcholine from *Buccinum undatum*[39]. Another group of natural products, terpenes, was isolated from ether extracts of dried specimens of sea hares *Aplysia kurodai*[40]: the bromine-containing aplysin and aplysinol, debromoaplysin and an optically active diterpene, aplysin-20[41]. It has been suggested that sea hares ingest these substances with their algal food, since the same compounds were isolated from marine algae of the genus *Laurencia*[42].





The venom of the Australian octopus *Hapalochlaena maculosa* contains one major toxic substance, designated 'maculotoxin' which is a highly polar, non-antigenic compound having a low molecular weight[43]. It appears to be chemically similar but distinct from tetrodotoxin (see: fish toxins). Eledoisin, a hypotensively active peptide consisting of 11 amino acid residues has been isolated from salivary glands of the Mediterranean octopod *Eledone moschata*[44,45]. Cephalotoxin, a toxic proteinaceous component, probably a glycoprotein, was also found in its glands and likewise in those of *Octopus vulgaris* and *O. macropus*[46].

5. *Echinodermata.* Sea cucumbers (*Holothurioidea*) contain in their body wall, but mainly in the Cuvierian glands extremely toxic substances which can be precipitated as a cholesterol complex and which have been characterized as steroid glycosides. CHANLEY et al.[47] isolated from the Bahamian sea cucumber, *Actinopyga agassizi*, holothurin A which has been found to be a mixture of sodium salts of sulfated aglycones linked to 4 sugar molecules. The sugars were indentified as D-xylose, D-glucose, 3-*O*-methyl-D-glucose and D-quinovose. For the parent substance,



the name holothurinogenin, a pentacyclic triterpene, has been proposed. Various closely related aglycones isolated from a number of holothurians can be deduced from this substance[48,49]. Asterosaponin A and B has been purified from the starfish *Asterias amurensis* and characterized as triterpenoid glycoside; however, the structure is still not completely elucidated[50].

6. *Vertebrata. Fishes.* Since ancient times it is known that various fishes belonging to the families *Tetraodontidae* and *Diodontidae* are extremely poisonous when eaten. The toxic principle, 'tetrodotoxin', has been isolated in crystalline form from the ovaries and liver of the puffer fish (fugu, *Sphoeroides rubripes*), its structure was elucidated by TSUDA et al.[51]. It is an amino perhydroquinazoline compound with an unique hemilactal link between two separate rings and a guanidinium group. The abundance of OH groups makes the molecule extremely polar. Tetrodotoxin has been found to be identical with tarichatoxin, which is present in eggs, ovaries and the skin of the Californian newt *Taricha torosa*[52], a rather surprising, unexplainable phenomenon.

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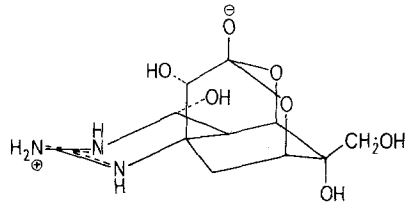
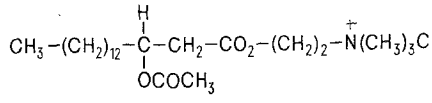
Ciguatera or tropical fish poisoning, due to the ingestion of various intermittingly toxic fishes such as snappers (*Lutjanidae*), groupers (*Serranidae*), moray eels (*Muraenidae*), surgeon fishes (*Acanthuridae*),



Tetrodotoxin

occurs predominantly in tropical areas of the globe[53]. Since ciguatera toxicity seems to be geographical in distribution and periodic in occurrence, it has been suggested that the primary source of the toxic principle accumulating in the fish body may derive from algae. A toxin extracted from the flesh of the moray eel *Gymnothorax javanicus* was characterized as a lipid containing a quaternary nitrogen atom, one or more hydroxyl groups and a cyclopentanone moiety[54].

In the mucous secretions of the Hawaiian box fish, *Ostracion lentiginosus*, a toxic substance named 'pahutoxin' has been identified as the choline chloride ester of 3-acetoxyhexadecanoic acid[55].

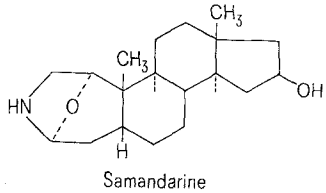


Pahutoxin

Fish toxins produced in special venom glands seem to be mostly proteins. The venom of the round stingray, *Urolophus halleri* has been reported to consist of proteins having molecular weights of more than 100,000[56]. Similar observations have been made with toxic material from the stonefish, *Synanceja trachynis* and *S. verrucosa*[57].

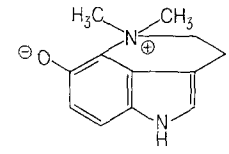
Amphibians. Amphibians such as frogs, toads, newts and salamanders produce in skin glands a large variety of pharmacologically active compounds of great chemical diversity including biogenic amines, peptides, proteins, steroids and alkaloids. Among these substances are some of the most powerful toxins known (see reviews[58–61]).

The identity of tarichatoxin from the North-American newts, *Taricha torosa*, *T. rivularis* and

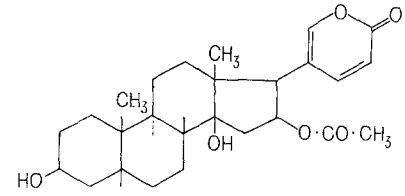


Samandarine

T. granulosa, with tetrodotoxin has already been mentioned. From skin secretions of salamanders (*Salamandra maculosa*), several alkaloids were purified and described as samandarine derivatives possessing an oxazolidine (samandarone etc.) or carbinolamine system (cycloneosamandione), or having none of these systems (samanine)[62]. Besides biogenic amines like adrenaline, noradrenaline and various indolalkylamines (bufotenines), toad poisons contain cardioactive steroids similar to the digitalis group, called bufogenins (bufotalin), and the conjugates of bufogenins with suberylarginine, bufotoxins[59,61]. Peptides



Dehydrobufotenine



Bufotalin

with a widespread spectrum of pharmacological activities such as hemolysis, blood pressure lowering, etc., were obtained from the skin of various frogs and toads like physalaemin from *Physalaemus fuscumaculatus*[63], caerulein from *Hyla caerulea*[64] and bombesin from *Bombina bombina*[65].

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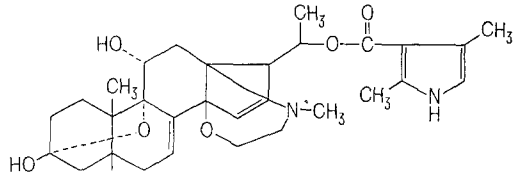
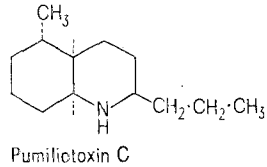
Steroidal alkaloids of high toxicity were isolated from skin extracts of frogs of the family *Dendrobatidae* and *Atelopidae*, which are used as arrow poisons by the Indians of the rain forest in Columbia. Batrachotoxin from *Phyllobates aurotaenia* has been identified as the major toxic component possessing many unusual structural features[66]. Pumiliotoxin A, B and C are three other toxins of a new alkaloid type isolated from *Dendrobates pumilio* and *Dendrobates auratus*. The structure of pumiliotoxin C has been elucidated[67]. Atelopidotoxin purified from *Atelopus zeteki* possesses similar high toxicity; its structure is still unknown[68].



Batrachotoxin



Pumiliotoxin C

Reptiles. Reptile venoms are a complex mixture of polypeptides and proteins possessing toxic and enzymatic properties (see reviews[69–73]). Besides the snakes there exists only one venomous lizard family, the *Helodermatidae* with two species *Heloderma suspectum* and *Heloderma horridum*. Their venom has phospholipase A, hyaluronidase, esterolytic and kinin-releasing activity and is remarkably toxic; a specific toxin has not yet been isolated[74].

Venoms of the snake families *Hydrophiidae* (sea snakes), *Elapidae* (cobras, kraits, mambas etc.), *Viperidae* (vipers), and *Crotalidae* (pit vipers, rattlesnakes) exhibit a wide spectrum of enzymatic action; however, with the exception of L-amino acid oxidase only, all other detectable enzyme activities are of hydrolytic type: hyaluronidase, phosphomono- and diesterase, nucleotidase, cholinesterase, phospholipase A, esterases (aliphatic and aryl-esterases), exo- and endopeptidases, blood clotting enzymes and enzymes releasing kinin from plasma precursors. Whereas venoms of *Crotalidae* and *Viperidae* snakes are especially rich in proteolytic enzymes, those of *Elapidae* possess almost no proteolytic but more esterolytic properties. *Hydrophiidae* venoms contain very few or no enzymes. Interestingly enough, snake venoms are one of the most concentrated enzyme sources in nature.

Neurotoxins producing curare-like symptoms and cardiotoxins are basic polypeptides with 60 to 74 amino acid residues predominantly found in *Hydrophiidae* and *Elapidae* venoms[73]. 'Cobrotoxin' from the Formosan cobra venom, *Naja naja atra*, was the first toxin of which the structure was elucidated[75]. In the meantime, more than 20 amino acid sequences of homologous toxins are known[76]. They all consist of a single polypeptide chain cross-linked by 4 or 5 disulfide bridges, cardiotoxins showing great structural similarities possess a higher content of lysine residues[77]. The integrity of the disulfide bonds is essential for the toxicity[78]. All toxins exhibit in their sequence common residues of which tyrosine and tryptophane seem to be important to maintain the molecule in its active conformation; basic residues might be essential for binding to the cholinergic receptor. More conclusive assumptions on structure-activity relationship will be obtained after synthesis of modified toxins. Recently the successful synthesis of cobrotoxin with 20% toxic activity has been reported[79].

Relatively few investigations deal with toxins from *Viperidae* and *Crotalidae* venoms. MOROZ et al.[80]

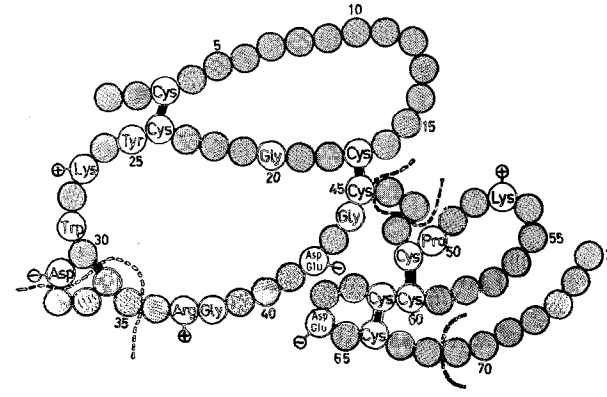


Fig. 1. Common structure of curare-like neurotoxins from *Hydrophiidae* and *Elapidae* venoms. The shadowed rings denote variant amino acids, those encircled by a dotted line are only found in toxins having 71 or 74 residues and are included in the structure of smaller toxins with 61 or 62 residues.

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Amino acid sequences of toxins and pharmacologically active peptides from animals.

Bee venom *(Apis mellifica)*

Mclittin[32]

Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln-NH_2

Apamin[23]

Cys-Asn-Cys-Lys-Ala-Pro-Glu-Thr-Ala-Leu-Cys-Ala-Arg-Arg-Cys-Gln-Gln-His-NH_2

MCD-peptide[67]

Ile-Lys-Cys-Asn-Cys-Lys-Arg-His-Val-Ile-Lys-Pro-His-Ile-Cys-Arg-Lys-Ile-Cys-Gly-Lys-Asn-NH_2

Wasp kinin[21] *(Vespa)*

Pyr-Thr-Asn-Lys-Lys-Lys-Leu-Arg-Gly-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg

Scorpion toxins[35,36] *(Androctonus australis)*

I. Lys-Arg-*Asp-Gly-Tyr-Ile-Val*-Tyr-Pro-Asn-*Asn-Cys*-Val-*Tyr*-His-*Cys*-Val-Pro-Pro-*Cys*-Asp-Gly-Leu-*Cys*-Lys-*Lys*-Asn-Gly-*Gly*-Ser-*Ser-Gly*-

II. Val-Lys-*Asp-Gly-Tyr-Ile-Val*-Asp-Asp-Val-*Asn-Cys*-Thr-*Tyr*-Phe-*Zys*-Gly-Arg-Asn-Ala-Tyr-*Cys*-Asn-Glu-Glu-*Cys*-Thr-*Lys*-Leu-Lys-*Gly*-Glu-*Ser-Gly*-

Ser-Ser-*Cys*-Phe-Leu-Val-*Pro*-Ser-*Gly*-Leu-*Ala-Cys*-Trp-*Cys*-*Lys*-Asp-*Leu-Pro-Asp*-Asn-*Val*-Pro-Ile-*Lys*-Asp-Thr-Ser-*Arg*-Lys-*Cys*-Thr

Tyr-*Cys*-Gln-Trp-Ala-Ser-*Pro*-Tyr-*Gly*-Asn-*Ala-Cys*-Tyr-*Cys*-Tyr-*Lys*-*Leu-Pro-Asp*-His-*Val*-Arg-Thr-*Lys*-Gly-Pro-Gly-*Arg*-*Cys*-His-NH_2

Eledoisin[45] *(Eledone moschata)*

Pyr-Pro-Ser-Lys-Asp-Ala-Phe-Ile-Gly-Leu-Met-NH_2

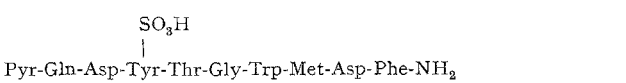
Amphibian skin peptides

Physalaemin[63] *(Physalaemus fuscumaculatus)*

Pyr-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met-NH_2

Caerulein[64] *(Hyla caerulea)*

Bombesin[65] *(Bombina bombina)*



Pyr-Gln-Asp-Tyr-Thr-Gly-Trp-Met-Asp-Phe-NH_2

Pyr-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2

Snake venom toxins

α-Bungarotoxin[88] *(Bungarus multicinctus)*

Cobrotoxin[75] *(Naja naja atra)*

Cardiotoxin[77] *(Naja naja atra)*

Ile-Val-*Cys*-His-Thr-Thr-Ala-Thr-Ile-Pro-Ser-Ser-Ala-Val-Thr-*Cys*-Pro-Pro-*Gly*-Glu-Asn-Leu-*Cys*-*Tyr*-Arg-Lys-Met-Trp-Cys-Asp-Ala-Phe-

Leu-Glu-*Cys*-His-Asn-Gln-Gln-Ser-Ser-Glu-Thr-Pro-Thr-Thr-Thr-Gly-*Cys*-Ser-Gly-*Gly*-Glu-Thr-Asn-*Cys*-*Tyr*-Lys-Lys-Arg-Trp-Arg-Asp-His-

Leu-Lys-*Cys*-Asn-Lys-Leu-Val-Pro-Leu-Phe-Tyr-Lys-Thr-*Cys*-Pro-Ala-*Gly*-Lys-Asn-Leu-*Cys*-*Tyr*-Lys-Met-Phe-Met-Val-Ala-Thr-

Cys-Ser-Ser-Arg-Gly-Lys-Val-Val-Glu-Leu-*Gly*-*Cys*-Ala-Ala-Thr-*Cys*-*Pro*-Ser-Lys-Lys-Pro-Tyr-Glu-Glu-Val-Thr-*Cys*-*Cys*-Ser-*Thr-Asp*-

Arg-Gly-Tyr-Arg-Thr-Glu-Arg-*Gly*-*Cys*-Gly-*Cys*-*Pro*-Ser-Val-Lys-Asn-Gly-Ile-Glu-Ile-Asn-*Cys*-*Cys*-Thr-*Thr-Asp*-

Pro-Lys-Val-Pro-Val-Lys-Arg-*Gly*-*Cys*-Ile-Asp-Val-*Cys*-*Pro*-Lys-Ser-Ser-Leu-Val-Leu-Lys-Tyr-Val-*Cys*-*Cys*-Asn-*Thr-Asp*-

Lys-*Cys*-*Asn*-His-Pro-Pro-Lys-Arg-Glu-Pro-Gly

Arg-*Cys*-*Asn*-Asn

Arg-*Cys*-*Asn*

purified a neurotoxin named 'viperotoxin' from the venom of *Vipera palaestinae* which has a molecular weight of about 11,600. From the venom of the South-American rattlesnake *Crotalus durissus terrificus*, crotamine, a strongly basic polypeptide with a molecular weight of about 5,500[81] and crotoxin with a molecular weight of 30,000[82] were isolated. The latter possessing beside toxicity also phospholipase A activity was separated into 2 components, a non-toxic, acidic polypeptide and a basic phospholipase A with very wol toxicity[83,84]. Only a combination of both factors restores the full toxic activity.

It has to be noted that the so-called non-venomous snakes of the family *Colubridae*, and probably of other families too, produce toxic secretions in homologous glands. Thus, the venom of the colubrid snake, *Dispholidus typus*, is highly toxic and possesses marked coagulant and proteolytic properties[85], that of *Leptodeira annulata* has phospholipase A, phosphodiesterase and proteolytic activity[86]. However, further research in this field may give a more comprehensive picture of these snake venoms in future.

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SPECIALIA

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Alkaloids of Cranberries V

We have recently shown[1-3] that extracts of cranberry leaves contain N-methylindolic[1] and N-methylazatricyclo type alkaloids[2,3]. Many studies have shown that most cranberry extracts (especially European) have an application in 'naive' cancer therapy as well as in traditional folklore medicine. New Brunswick cranberry extracts were purified and a basic fraction separated[2], using column chromatography fractionation. Final purification was realised by thin-layer preparative chromatography, giving a minimum of 19 different basic products. We have succeeded in isolating and identifying the three principal constituents, and now report the configurational and conformational results of our work.

Method. Three products – cannivonines **1**, **2** and **3** – were isolated from 2.5 kg of dry material, with respective yields of 1.7, 5.3 and 1.4 mg[1,2].

Results and discussion. The mass peaks of the products studied by high resolution mass spectrometry (AEI

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Table I. Cannivonine 2

Chemical shifts				Vicinal coupling Hz		
	δ	δSR[a]	J	HC ∅°	B ∅°	Observed[a]
OH	3.60	4.20	6.7	4.3 (45)	9.1 (0)	4.5
CH–OH	4.20	5.16	7.11	7.9 (20)	2.5 (100)	7.0
N–CH_3	3.71	4.02	1.11	2.8 (55)	2.0 (60)	2.5
CH_3–CH_2	1.65	1.85	7.8	1.3 (65)	5.1 (40)	1.0
CH_2–CH_3	1.02	1.15	5.6	2.5 (80)	5.1 (40)	2.0
CH_3–C=	2.3	2.84	4.5	—	—	10.2
H–11	2.0–2.3	2.53	3.4	5.1 (40)	7.2 (20)	4.5
H–5	5.5–5.6	6.03				
H–4		5.84	10.9 ax	7.2 (25)	7.2 (20)	7.0
H–3	2.0–2.3	2.6	10.9 eq	5.9 (145)	2.5 (100)	5.0
H–1		2.5	1.10	2.5 (80)	2.0 (60)	2.2
(CH=CH–C)–11	4.7	5.3 and 5.5				

[a] After addition of 0.1 m E-FOD. Varian HR 220 MHz.