Pure & Appl. Chem., Vol.54, No.10, pp.1981-1994, 1982.
Printed in Great Britain.

0033-4545/82/101981-14$03.00/0
Pergamon Press Ltd.

BIOLOGICALLY ACTIVE COMPOUNDS FROM COELENTERATES

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Abstract - Many coelenterate species produce toxins in order to capture prey or for defence. Most of them are polypeptides and proteins excepting palytoxin, lophotoxin and a few physiologically less active organic compounds e.g. tetramine, serotonin and histamine. Coelenterates also contain other biologically active compounds e.g. prostaglandins with hormone-, cembrene derivates with antitumor activities and proteinase inhibitors. The present state of knowledge on the most important biologically active compounds from coelenterates is reviewed and a new method for the isolation of the caribbean palytoxin (C-PTX) is described.

INTRODUCTION

Coelenterates are simple metazoans which are composed essentially of two epithelial layers (ectoderm and entoderm) and one internal cavity (coelenteron) opening only at the mouth. Most of them possess tentacles equipped with stinging organelles (nematocysts) containing toxins.

The phylum Coelenterata is subdivided into three classes (Hydrozoa, Scyphozoa and Anthozoa) including about 9000 known species of which 70 are reported to be injurious to man (Ref. 1). The stinging abilities of coelenterates are said to have been known to Aristotle (Ref. 2). The investigation of their toxins began by Richet with the extraction of two toxic compounds (congestin and thalassin) from the tentacles of the sea anemone Anemonia sulcata (Ref. 3). After repeated injections of sublethal doses of the anemone toxin, congestin, into dogs, Richet discovered incidentally the phenomenon of anaphylaxy (Ref. 4). Since that time, the isolation and characterization of biologically active compounds from coelenterates has become of interest.

It is beyond the scope of the present work to refer to all the extensive efforts which have been achieved until now on the isolation and characterization of toxins and other biologically active compounds from coelenterates. This is the subject of previous review articles (Ref. 5-22).

The aim of the present work is to briefly summarize the most significant efforts which have been achieved up to now on the isolation and characterization of biologically active compounds from the diverse species of the three classes of the phylum Coelenterata, focussing particular attention on the individual origin, chemistry and scientific importance of the coelenterate toxins.

CHEMISTRY

Based on their chemical structure, we can differentiate between the following types of biologically active compounds isolated from coelenterates: quaternary ammonium compounds (e.g. tetramine, anthopleurine†), biogenic amines (e.g. histamine. serotonin, dopamine), N-methyl-pyridinium betains (e.g. trigonelline), long -chain aliphatic unsaturated carboxylic acids and their methyl esters (e.g. prostaglandins and their precursors), cyclic diterpene-(cembrene) derivatives (e.g. sarcophine, sinularin, crassin acetate, eunicin, lophotoxin), 1,3,5,7- and 1,3,7,9- tetrazacyclopent- azulens (zooanthoxanthin, paragracine), polypeptides (e.g. neuro- and cardiotoxins and proteinase inhibitors), proteins (e.g. hemolysins, cardiotoxins, enzymes) and polyketid-like polyhydroxy-compounds (palytoxins).

†Table 2

TABLE 1. Classification of selected coelenterate species containing toxins or other biologically active compounds

Class	Order	Family	Species	References
HYDROZOA	Athecata	Milleporidae	Millepora a.	23
(2700 species)			Millepora t.	24,25
	Siphonophora	Physaliidae	Physalia p.	9,26-28
SCYPHOZOA	Cubomedusae	Chirodropidae	Chironex f.	9,29-31
(200 species)			Chiropsalmus q.	9,32
	Semaestomae	Pelagiidae	Chrysaora q.	9
			Pelagia n.	33
		Cyaneidae	Cyanea c.	34,35
		.....	Stomolophus m.	9,36,37
ANTHOZOA (6100 species)				
Hexacorallia	Madreporaria	.....	Goniopora sp.	15,38,39
	Zoantharia	.....	Palythoa c.	47,48
			" m.	47
			" tox.	15,43,44
			" tub.	15,45,46
			" v.	49
			Parazoanthus a.	17,40,41
			" g.	18,42
	Actinaria	Family groups		
		Abasilaria	Actinia e.	50,51
		Boloceroidaria	Actinodendron p.	20
		Endomyaria		
		Mesomyaria	Aiptasia p.	52,53
			Anemonia s. -	11,13,20,54-61
			Anthopleura e.	19,62,63
			" x.	19,63,64
			Bolocera t.	65,66
			Condylactis a.	11,13,67
			" g.	68-70
			Epiactis p.	71
			Homostichantus d.	72,73
			Parasicionis a.	74
			Stoichactis g.	20,75
			" h.	76
Octocorallia	Alcyonaria	Alcyoniidae	Sarcophytum g.	77
	Gorgonaria	.....	Eunicia m.	12,14,81
			Lophogorgia sp.	82
			Plexaura h.	78,79
			Pseudoplexaura sp.	12,14,81
			Sinularia f.	12,14,80

Low molecular weight compounds

The symptoms of pain, erythema and edema at the site of nematocyst stings led to the early studies on low molecular weight compounds (Mw < 500) of coelenterate toxins (Ref. 83-85 and Table 2). From these compounds only serotonin has received particular attention because of its pain-producing and histamine-releasing properties (Ref. 86).

In 1969, Weinheimer and Spraggins reported on the occurrence of two new prostaglandin derivates in the gorgonian Plexaura homomalia (Ref. 78,79). The dry cortex of this horny coral contains 0.2% 15-epi-PGA_2 (see Table 2) and 1.3% of its O-acetyl-methylester. These compounds were biologically inactive, however they could be converted chemically into the highly active $PGF_2\alpha$ (Ref. 87). The prostaglandins are a group of C_{20} unsaturated carboxylic acids with a wide range of hormone-like activities. Their biological activities vary with very slight changes in their structure (Ref. 88).

Common metabolites of sea fans (Gorgonaria) and of soft corals (Alcyonaria) are several cembrene-lacton derivatives (cembrenolides) with cytotoxic and antineoplastic activity (Ref. 12,14,89). Their basic cembrene skeleton is a 14-membered diterpenoid generated by the cyclization of geranyl-geraniol between the carbon atoms 1 and 14. A series of new nitrogen-containing tricyclic metabolites with a tetrazacyclopent-azulen skeleton have been isolated recently from several zooanthid species (Ref. 19,40-42). Two of these compounds, zooanthoxanthin and paragracine, were found to have well-defined biological activities: the former inhibiting DNA synthesis (Ref. 90) and the latter displaying papaverine-like spasmolytic properties (Ref. 42).

The quaternary ammonium compound anthopleurine (Table 2), isolated from the sea anemone Anthopleura elegantissima, proved to be an alarm pheromone (Ref. 91). It is released by an injured member causing other members of the sea anemone colony (A. elegantissima) to contract.

Middle and high molecular weight compounds (Mw 2000-300 000)

Almost all coelenterate toxins are polypeptides or proteins which vary considerably in both chemical and physical properties among the coelenterate species (Ref. 8,9,11,13,19,20,21), the palytoxins being an exception (Ref. 43,45, 47).

The interest in coelenterate toxins increased considerably in 1968 when Shapiro published his results on the purification of the first toxic polypeptide isolated from a sea anemone (Condylactis gigantea), and when the specific action of this toxin on the sodium channel was discovered (Ref. 68,102)

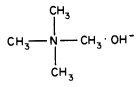
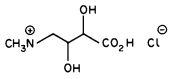
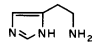
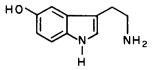
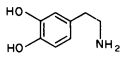
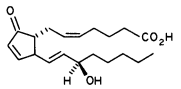
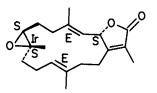
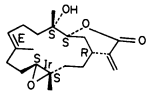
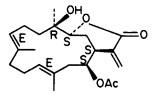
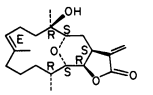
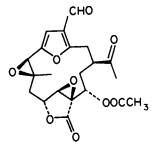
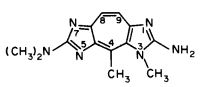
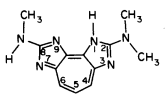
Shortly thereafter, two systematic studies began independently of one another in 1971 on the isolation and characterization of toxins from two further coelenterate species, in particular from the sea anemone Anemonia sulcata (Ref. 54,55,103) and from the zooanthid Palythoa toxica (Ref. 43,104). These studies resulted in the complete purification of the sea anemone toxins (I,II and III) and of the palytoxin in larger amounts enabling the structure elucidation and extensive physiological and pharmacological investigations.

In spite of the phylogenic relation of these two coelenterate species (Anemonia sulcata and Palythoa toxica), the toxins isolated from them proved to be chemically completely different types of compounds: polypeptides in the case of the sea anemones and a polyketid-type polyhydroxy compound in the case of the palythoa specimens.

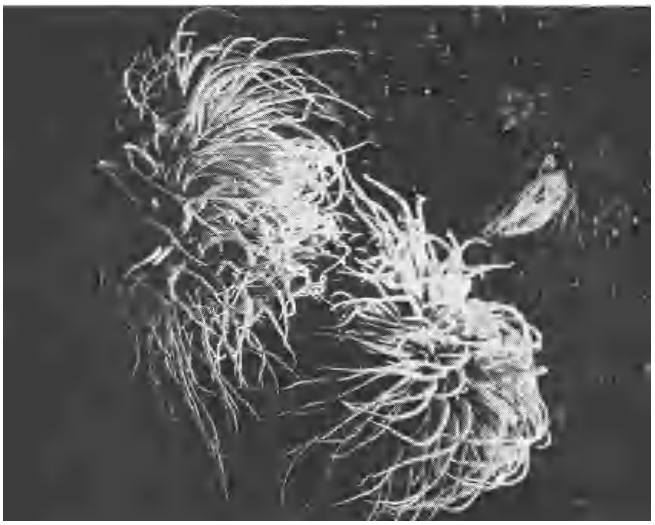
Altogether twenty two sea anemone toxins and five palytoxins from different sea anemone- and palythoa specimens respectively have been isolated since 1971 (Ref. 20,45,47,49,50,62,64,66,67,69,70-72,75). All the sea anemone toxins proved to be polypeptides or proteins with molecular weights ranging either from 2000-5500 or from 10,000-25,000 Daltons. The isolated palytoxins were found to be nonprotein-polyhydroxy compounds. They fall under the category of polyketides (Ref. 44,46).

The toxins isolated from species of the classes Hydrozoa and Scyphozoa are exclusively proteins with molecular weights higher than 10,000 Daltons. All of these proteins are very labile compounds. In spite of intensive research efforts (Ref. 8,9), no toxin of these groups could be completely purified up to now.

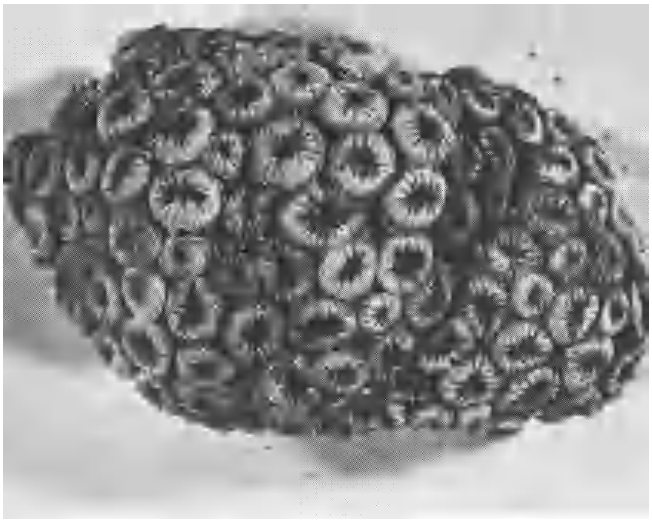
TABLE 2. Biologically active compounds of low molecular weight (Mw<500)

Compound	Structure	Isolated from:	Biological activity	Reference
Tetramethyl-ammonium·OH		Actinia e.	ganglion blocker LD_{50} 30 mg/kg (mice)	83,84,92, 93
Anthopleurine[+]		Anthopleura e.	alarm pheromone	91,94
Histamine		Anemonia s. Actinia e.	biogenic amine e.g. vasodilatator LD_{50} 12 mg/kg(mice)	84
Serotonin		Anemonia s. Metridium s. Chrysaora q.	biogenic amine e.g. vasoconstrictor LD_{50} 160 mg/kg(mice)	16,84,95
Dopamine		Metridium s.	neurotransmitter	16,96
Trigonelline		Anemonia s.	LD_{50} 4.8 mg/kg(mice)	92,97
Prostaglandin (15-epi-PGA_2)		Plexaura h.	no activity, but precursor of $PGF_2\alpha$	78,79,87
Sarcophine		Sarcophiton g.	toxic for fish	98
Sinularin		Sinularia f.	cytotoxic, antineoplastic	80,89
Crassin acetate		Pseudoplexaura p.	cytotoxic, antineoplastic	99,100
Eunicin		Eunicea m.	cytotoxic, antineoplastic	12,89,101
Lophotoxin		Lophogorgia sp.	neuromuscular blocker LD_{50} 8 mg/kg(mice)	82
Zooanthoxanthin		Parazoanthus a.	inhibits DNS synthesis	90
Paragracine		Parazoanthus g.	spasmolytic	42

[†]Not to be confused with the heart stimulant polypeptides -A; -B; -C (Ref. 63,64).



Anemonia sulcata



Palythoa caribaeorum

Toxic polypeptides and proteins

According to their molecular weight, we can differentiate between three groups of toxic polypeptides isolated from coelenterates: one group within the molecular weight range of 2000-3000 Daltons, a second group within the molecular weight range of 4000-6000 Daltons and finally a third group of toxic proteins having molecular weights over 10,000 Daltons (Table 4).

The first group includes only three sea anemone toxins: the toxins III and IV from the sea anemone Anemonia sulcata (Ref. 13,57) and the toxin from the sea anemone Parasicyonis actinostoloides (Ref. 74). From these three toxins, only the toxin III and toxin IV from Anemonia sulcata have been chemically characterized. The toxin III (ATX III) contains twenty seven amino acids including 6 cysteins which are interconnected by 3 disulfide bridges (Ref.57) The toxin IV (ATX IV) from Anemonia sulcata is a degradation product of toxin III which lacks the latter two amino acids of toxin III: valine and lysine (Ref. 13,105).

The second group of toxic polypeptides presently include fifteen analytically pure and three partially purified sea anemone toxins(Table 4).They generally contain six cystein residues, an exception being the sea anemone Actinodendron plumosum which is reported to have only four cysteins (Ref. 20). The presence of four cystein molecules was first reported in the toxin APB of the sea anemone Anthopleura xanthogrammica (Ref. 63); this was however later corrected to six cysteins (Ref. 20).

Members of this group of sea anemone toxins represent a new class of polypeptide toxins with a high degree of homology in their amino acid sequences. The sequences of five sea anemone toxins are currently known (Ref. 56,57,59, 63,106), also including that of the smaller molecular ATX III from the first group which is an exception as no evident homology to the sequences of the other four toxins can be detected (Table 3). All primary structures have charged polar groups at their ends. The inner part of the sequences contain hydrophobic amino acids in position 2-6 and 21-31 in ATX I, ATX II, APC and APA and in position 3-19 in ATX III. The position of the three disulfide bridges of two of these toxins(ATXII and APA)has been determined as: cys^{4}-cys^{44}, cys^{6}-cys^{34} and cys^{27}-cys^{45} by Wunderer (Ref. 60) and cys^{6}-cys^{36}, cys^{4}-cys^{46} and cys^{29}-cys^{47} by Yasunobu et al. (Ref. 19,107) respectively. They are paired to each other in a very similar fashion in both toxins.

TABLE 3. Sequences of five sea anemone toxins

	1	5	10	15	20	(25)	(29)
ATX I:	Gly	Ala	Ala/Pro	Cys Leu Cys	Lys	Ser Asp Gly Pro	Asn Thr	Arg Gly Asn	Ser Met	Ser Gly	Thr	Ile Trp	Val	Phe	Gly Cys
ATX II:	Gly	Ile/Val	Pro Cys Leu Cys Asp Ser Asp Gly Pro Ser Val Arg Gly Asn Thr Leu Ser Gly Ile Ile Trp Leu											Ala	Gly Cys
AP - C:	Gly Val Pro Cys Leu Cys Asp Ser Asp Gly Pro Ser Val Arg Gly Asn Thr Leu Ser Gly	Ile	Leu	Trp Leu										Ala	Gly Cys
AP - A:	Gly Val	Ser	Cys Leu Cys Asp Ser Asp Gly Pro Ser Val Arg Gly Asn Thr Leu Ser Gly	Thr Leu	Trp	Leu Tyr Pro Ser	Gly Cys								
ATX III:	Arg Ser Cys	Cys	Pro	Cys	Tyr Trp Gly	Gly	Cys Pro Trp Gly Gln	Asn	Cys Tyr Pro Glu Gly Cys Ser Gly					Pro Lys Val	

	(30)	(35)	(40)	(45)	(49)
ATX I:	Pro Ser Gly Trp	Asn	Asn Cys	Glu Gly Arg Ala	Ile	Ile Gly Tyr Cys Cys Lys Gln
ATX II:	Pro Ser Gly Trp His Asn Cys Lys	Lys	His Gly Pro Thr Ile Gly Trp Cys Cys Lys Gln			
AP - C:	Pro Ser Gly Trp His Asn Cys Lys Ala His Gly Pro Thr Ile Gly Trp Cys Cys Lys Gln					
AP - A:	Pro Ser Gly Trp His Asn Cys Lys Ala His Gly Pro Thr Ile Gly Trp Cys Cys Lys Gln					

ATX I,II,III : Toxins from Anemonia sulcata (Ref. 56-59)
APA : Toxin from Anthopleura xanthogrammica (Ref. 106)
APC : Toxin from Anthopleura elegantissima (Ref. 63)

Natural abundance ^{13}C-NMR studies of the toxins APA and ATX II indicated recently, that their overall conformation is very similar (Ref. 108,109). APA is known to have a compact structure containing numerous ß-bends and some ß-pleated sheet regions (Ref. 110).

TABLE 4. Toxic polypeptides and proteins isolated from coelenterates

Coelenterate species	Number of isolated polypeptides/proteins			Mode of action	Purity	References
	Mw: 2000-3000	Mw: 4000-6000	Mw: 10,000-300,000	LD_{50}/mice	+ pure - impure	
Millepora a.			1	respiratory distress LD_{50} 40/ug/kg	-	23
Millepora t.			1	hemolytic, dermonecrotic LD_{50} 38/ug/kg	-	24,25
Physalia ph.			2 + 8	dermonecrotic, cardiotoxic LD_{50} 70/ug/kg	-	8,9
Chyronex fl.			2	cardiotoxic, hemolytic dermonecrotic	-,-	8,9
Chrisopsalmus q.			2	hemolytic, dermonecrotic, cardiotoxic	-,-	8,9
Chrysaora q.			25	dermonecrotic, cardiotoxic, fibrinolytic	-	8,9,124
Cyanea c.			1	dermonecrotic, cardiotoxic LD_{50} 300/ug/kg	-	34,35
Stomolophus m.			1	dermonecrotic, cardiotoxic	-	8,9
Goniopora sp.			1 + 1	1x cardiotoxic, 1x hemolytic LD_{50} 300/ug/kg	+,-	15,39
Actinia e.			1	hemolytic, cardiotoxic LD_{50} 33/ug/kg	+	50,123
Actinodendron p.		1		neurotoxic LD_{50} 6 mg/kg	+	20
Aiptasia p.			2	hemolytic, neurotoxic	-,-	8,53
Anemonia s.	2	3		neurotoxic, cardiotoxic LD_{50} for ATX II 300/ug/kg, 100/ug/kg	+,+,+ +,+	11,20, 121,143
Anthopleura e.		1		neurotoxic, cardiotoxic	+	19
Anthopleura x.		2		neurotoxic, cardiotoxic LD_{50} for APA ca.400/ug/kg	+,+	125-130
Bolocera t.		2	1	2x neurotoxic, 1x hemolytic	+,+ -	66 65
Condylactis a.		4		neurotoxic	+,+,+,+	67
Condylactis g.		2	1	2x neurotoxic 1x " , hemolytic	-,- +	69 68,70
Epiactis p.			3	hemolytic	+,+,+	71
Homostichantus d.		1		neurotoxic, cardiotoxic	+	72,73
Parasicionis d.	1			neurotoxic	-	74
Stoichactis g.		2		neurotoxic, cardiotoxic LD_{50} 2 mg/kg	+,-	20,75
Stoichactis h.			1	hemolytic	+	76,119, 131,132

By means of specific chemical modifications, toxicity tests on crabs and mice and binding studies to the Na^+-channel of rat brain synaptosomes, the structure-function relationship of ATX II from Anemonia sulcata has been established (Ref. 111). The most striking result of this study was the fact that modification of the carboxyl groups of both aspartate residues and that of the C-terminal, resulted in the complete loss of toxicity, but not of the binding properties of the modified ATX II. The modification of the single arginin residue of ATX II in position 14, led to the complete loss of both toxicity and binding ability. The same is true in part for the acetylation of the free amino groups of the two lysin residues and that of the terminal glycin (Ref. 111).

Radioactive labeling of the sea anemone toxins ATX I and ATX II was first reported by Hucho and coworkers (Ref. 112). ATX I was iodinated by a slight modification of the lactoperoxidase method (Ref. 113,114) and ATX II was tritiated by reductive alkylation via the Shiff base formed by pyridoxal phosphate and amino groups of the peptide toxin (Ref. 115). The toxicity of the samples ATX I and ATX II remained unchanged after these procedures. In addition, the modification of the histidine residue of ATX II with ^{125}I did not alter the biological activity when monoiodination was achieved (Ref. 116, 117). In the case of diiodination, the activity of the modified ATX II was reduced by 75% (Ref. 116).

The third group of toxins includes the toxic proteins of widely different molecular weights ranging from 10,000 up to 300,000 Daltons. Purified compounds from this group are rare only including some hemolytic toxins of molecular weights of about 20,000 Daltons isolated from different sea anemone species e.g.: Aiptasia pallida (Ref. 52,53,118), Actinia equina (Ref. 50,51), Stoichactis helianthus (Ref. 76,119,120), Bolocera tuediae (Ref. 65), Condylactis gigantea (Ref. 70), Epiactis prolifera (Ref. 71) and one of the two goniopora toxins (Mw =12,000) isolated from the coral Goniopora sp. (Ref. 15, 38). The gonioporatoxin was reported to have cardiotoxic properties (Ref. 39) similar to those of ATX II from Anemonia sulcata (Ref. 121,122).

Proteinase inhibitors

Sea anemone species also contain polyvalent proteinase inhibitors in relatively high concentrations. These were first detected in Anemonia sulcata (Ref. 54) and later in other sea anemones (Ref. 133). Five isoinhibitors from Anemonia sulcata were isolated and characterized (Ref. 134). In a later work, the isolation of five additional isoinhibitors from the same batch was described (Ref. 135). All of these proteinase inhibitors are polypeptides with molecular weights of about 6500 Daltons. The sea anemone proteinase inhibitors are the first homologues to the Kunitz Inhibitor (BPTI) found in nature (Ref.136). In contrast to other biologically highly active coelenterate polypeptides, they are not toxic. Because of the capability of all of these proteinase inhibitors to inhibit trypsin, chymotrypsin, serum- and organokallikreins and plasmin, the inhibition spectrum of the sea anemone proteinase inhibitors is similar to that of the Kunitz Inhibitor (Ref. 134). The latter is well known for its antiinflammatory properties (Ref. 137). However , the metabolic pathway of these two types of proteinase inhibitors is different; whereas the Kunitz Inhibitor (BPTI) is quickly bound in the kidney (Ref. 138), the sea anemone inhibitor (SAI) remains in circulation and is secreted in the urine (Ref. 139).

The proteinase inhibitors of the sea anemone Anemonia sulcata can be isolated simultaneously with its toxins using the same techniques (Ref. 55).

A NEW METHOD FOR THE ISOLATION OF THE CARRIBBEAN PALYTOXIN (C-PTX)

For over ten years, we have been involved in the isolation and characterization of toxic polypeptides from sea anemones. Recently we wanted to know whether our elaborated techniques for the purification of biologically active polypeptides from sea anemones (Ref. 13,54,55,66,67,134) could also be used with a slight modification for the isolation of another type of water soluble coelenterate toxin of higher molecular weight, in particular for the caribbean palytoxin (Ref. 47).

Toxin isolation from Palythoa caribaeorum was achieved by the extraction of the toxin with 50% ethanol from the homogenized specimens, gel filtration on Sephadex G 50 and ion exchange chromatography on QAE- and SP-Sephadex. Final purification was obtained by gel filtration on Biogel P 6 (Ref. 48).

TABLE 5. Isolation steps of Palytoxin (C-PTX). Method I (Ref.48).

2 kg lyophilized P. caribaeorum
15 l 50% ethanol. Homogenisation. Filtration.

ETHANOLIC EXTRACT: (toxin $40x10^6$ CU§)
concentration to 500 ml at reduced pressure + 5 l acetone → RESIDUE (discarded)

SUPERNATANT → PRECIPITATE: (toxin)
concentration to 50 ml at reduced pressure + 100 ml H_2O + 300 ml ether separatory funnel

+ 300 ml 0.2 M acetic acid
centrifugation (12.000 g)

SUPERNATANT: (toxin $40x10^6$ CU)
gelfiltration on Sephadex G 50 (in 0.1 M acetic acid)

WATER LAYER	ETHER LAYER
+ 200 mg charcoal filtration lyophilization	lipoid compound etheric oils

FRACTION 3: (toxin $36x10^6$ CU)
lyophilization, chromatography on QAE-Sephadex A 25 in 0.01 M Tris-HCl buffer pH=8

15 g water soluble nontoxic compound

FRACTION 1: (toxin $30x10^6$ CU)
ultrafiltration on Amicon UM 05

FILTER RESIDUE: (toxin $28x10^6$ CU)
1/2 part chromatography on SP-Sephadex C-25 in 0.1 M Na-acetate buffer pH= 4.5; 1/2 part chromatography on CM-cellulose NH_4-acetate buffer pH=6

FRACTION 2: (toxin $25x10^6$ CU)
lyophilization, gel filtration on Biogel P 6 (200-400 mesh)

§ CU = crab unit

FRACTION 2: Palytoxin (C-PTX)
lyophilized 60 mg = $24x10^6$ CU
total yield 60%

The advantage of this method over previous techniques (Ref. 43,45,47,140) is the use of gel filtration on Sephadex G50 in the first chromatographic step enabling a highly specific purification of the toxin already at the beginning of the purification procedure. The gel filtration on Biogel P6 is indispensable in order to obtain pure palytoxin C-PTX . The UV- and IR-Spectra of the pure toxin (Ref. 48) proved to be indentical to those of the previously isolated palytoxin (Ref. 47).

SEA ANEMONE TOXINS AND PALYTOXIN AS TOOLS FOR PHYSIOLOGICAL, PHARMACOLOGICAL AND BIOPHYSICAL RESEARCH

The nerve impulse (action potential) is generated by the flow of sodium and potassium ions through molecular channels (Na^+ and K^+ channels) embedded in the nerve membrane. Any chemical influences on these channels (or the generation of new channels) consequently has a rigorous effect on the nerve conduction. Several toxic natural products (e.g. veratridine, aconitine, batrachotoxin, grayanotoxin, saxitoxin and tetrodotoxin) exert their potent physiological effect by modifying the voltage-sensitive Na^+ channels which are involved in the action potential generation in the nerve, heart and skeletal muscles (Ref. 141). With the isolation of the sea anemone toxin ATX II (and other sea anemone toxins) in our laboratory, the research efforts of cooperating electrophysiologists (Ref. 142-146) and through independent contemporary studies (Ref. 147,148), ATX II was found to be a new tool for the highly specific modification of the sodium channel. It acts on the H-gate of the sodium channel by slowing down the inactivation (Ref. 144,145,148); a process which gives rise to the prolongation of the action potential duration (Ref. 142,143) and to the enormous increase of transmitter release from nerve terminals (Ref. 148,149). The pure sea anemone toxin ATX II was also found to be

cardiotoxic evoking a dose-dependent, positive inotropic effect even in nanomolar concentrations (Ref. 121,150). The same effect on the mammalian heart was also reported later for the sea anemone toxin APA which was found twice as potent as ATX II (Ref. 125,126). The positive inotropic effect of ATX II on the mammalian heart was accompanied by a prolongation of the action potential duration (Ref. 122).

The high affinity of sea anemone toxins for the fast sodium channel, specifically on the inactivation process, and its action on the mammalian heart renders these toxins important tools in the study of the sodium channel (Ref. 19, 151-157). ATX II is already commercially available (FERRING GmbH, Wittland 11, 23 Kiel, West Germany).

Apart from certain protein toxins (e.g. botulinus- or tetanus toxin), the palytoxins isolated from marine zooanthid species (genus Palythoa) are the most toxic substances known (Ref. 158-162). In mammals, the toxicities (LD_{50}) of the palytoxins isolated from different palythoa species range from 0.025-0.6 µg/kg after parenteral application (Ref. 43,45,47,158,161) exceeding the average toxicity of the sea anemone toxin ATX II, LD_{50}=300 µg/kg mice by a factor of over 1000.

In appropriate concentrations, palytoxins force every contractile organ into contracture (Ref. 158,159,162-167) causing a general depolarisation by increasing the sodium permeability (Ref. 163,166). Since the raised sodium permeability through the excitable membranes is only partially suppressed by tetrodotoxin, a general membrane damage induced by the toxin rather than a specific action on the sodium channel may be assumed (Ref. 166,168).

Palytoxin proved to be in fact a potent hemolysin, which has a slow course of action and also causes a large prelytic potassiun loss in erythrocytes (Ref. 168). It induces the release of histamine from rat mast cells and is presently the most potent histamine releaser (Ref. 169). Palytoxin forms small pores in the red cell membranes raising their permeability to Na^+, K^+ and cholin, but not to Ca^{++}, sucrose or inulin (Ref. 170). Ouabain in concentrations as low as $5x10^{-8}$ M completely prevents the palytoxin effect (Ref. 171). The palytoxin is an extraordinary toxin not only because of its potency, but also for its interaction with ouabain on the erythrocyte membrane (Ref. 171).

Palytoxin seems to be developing into a new tool for membrane research (Ref. 172).

Aknowledgements- The author is particularly grateful for the fruitful discussions with Dr. Carsten Alsen. Thanks is also due to Mr. Joachim Zwick for technical assistance and Dipl. Biologist Mrs. Rachel Wolff for correction of the manuscript. Financial support was provided by a grant from the Deutsche Forschungsgesellschaft (Be 554/13).

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