Reference: *Biol. Bull.* **183:** 159–164. (August, 1992)

Conus Peptides: Phylogenetic Range of Biological Activity

LOURDES J. CRUZ[1,2], CECILIA A. RAMILO[1]*, GLORIA P. CORPUZ[1], AND BALDOMERO M. OLIVERA[2]

[1]*Marine Science Institute, University of the Philippines, Diliman, Quezon City 1101, Philippines, and* [2]*Department of Biology, University of Utah, Salt Lake City, Utah, 84112*

Abstract. The major function of the venoms of the predatory marine snails belonging to the genus *Conus* is to paralyze prey. Thus, the venom of each *Conus* species acts on receptors and ion channels of the prey; previous studies suggested much less activity on homologous receptor targets in more distant taxa. In this article, we address the question of whether some peptide components of *Conus* venoms ("conopeptides") have "cross-phylum" biological activity.

We examined the venom of *Conus textile,* a mollusk-hunting *Conus,* using a mammalian biological activity assay. We purified a 23 amino acid "convulsant peptide" with potent activity in the mammalian CNS, even though it comes from the venom of a snail-hunting *Conus* species. A survey of *Conus textile* venom fractions indicates that, in addition to the convulsant peptide, many other components of this venom will exhibit "cross-phylum" biological activity. Conopeptides with broad-range phylogenetic specificity should be useful tools for studying the evolution of receptors and ion channels, and of nervous systems.

Introduction

The small peptides present in the venoms of the predatory marine snails that belong to the genus *Conus* (cone snails) are proving to be of great interest in molecular neuroscience. This large and diverse family of highly constrained peptides ("conopeptides") are 10–30 amino acids in length and exhibit exquisite specificity towards cell surface receptors and ion channels (for recent overviews see Olivera *et al.,* 1990, 1991). Some of the peptides are now choice research tools in neurobiology. Because they are relatively rigid, their three-dimensional structures can be determined; the structures of several conopeptides have been solved by multidimensional NMR techniques. Given their small size and constrained conformation, the *Conus* peptides have great potential as lead compounds for drug development.

In this introduction, we will briefly review the biology and biochemistry of conopeptides. Furthermore, we describe results on a peptide that has "cross-phylum" biological activity. Such conopeptides may be useful tools for examining how certain receptors and ion channels have been used by various phyla in the course of evolution.

Biology and biochemistry of conopeptides

The cone snails are believed to be the most venomous of all molluscs. Many human fatalities have been caused by cone snail envenomation. There are about 500 *Conus* species; all are predators, and all use venom as the primary means for subduing prey. The venom is injected into the prey with a hollow harpoon-like tooth. The species of *Conus* can be divided into three major groups on the basis of the animals they feed on, *i.e.,* the worm-hunting, mollusk-hunting and fish-hunting Conidae. The venom of at least one species of each of these groups has been analyzed.

A major factor that contributes to the success of *Conus* is the remarkable biochemistry of their venoms. The biologically active components of *Conus* venoms are an exceptionally diverse family of small, conformationally constrained peptides. A single *Conus* venom may well have over fifty different such peptides, with varied pharmacological specificities. From the numerous peptides that have been purified from various *Conus* species, certain general concepts have emerged.

Each natural conopeptide appears to be specifically targeted to a macromolecular receptor, interfering with its

Received 7 April 1992; accepted 29 May 1992.

* Present address: Department of Chemistry, Washington State University, Pullman, Washington.

normal function. The conopeptides can have very high affinity for their receptors: ω-conotoxin GVIA, for example, targets to neuronal voltage-sensitive calcium channels and has sub-picomolar affinities for the high affinity targets. The high affinity and specificity of conopeptides is dependent on their highly constrained, relatively rigid conformations. In the majority of conopeptides, Cys residues, which are involved in forming multiple disulfide bonds, constitute 20–50% of all amino acids (for an overview, see Olivera *et al.*, 1990).

Peptides in the size range of the conopeptides would not normally have a specific conformation, but would equilibrate between many alternative conformations under physiological conditions. Usually, about 50 amino acid residues would be required before a polypeptide assumed a specific conformation. The sum of noncovalent forces (hydrogen bonds, hydrophobic interactions, and the like) in a small peptide are insufficient to stabilize a single conformation. But in most conopeptides the covalent cross-linking through multiple disulfide bonding presumably stabilizes the biologically active conformation. Indeed, conopeptides have some of the highest known densities of disulfide bonding found in any biological system; one 12 amino acid peptide isolated from a *Conus* venom has three disulfide bonds (see Olivera *et al.*, 1990).

A recent examination of cDNA clones encoding various conopeptides has revealed how the cone snails may have evolved the complex pharmacological cocktails of conopeptides that are their venoms. As will be described elsewhere (D. R. Hillyard, unpub. results), an "antibody-like evolutionary strategy" probably generated the array of small *Conus* peptides with diverse ligand specificity, but relatively conserved folding pathways and structural frameworks. The net result is that every species has its own characteristic mixture of peptides, and this conopeptide cocktail is highly potent on the particular prey type on which the species specializes. Thus, not only has a rather unusual biochemical strategy been used for paralyzing prey (*i.e.*, small, highly constrained peptides), but to generate the wide diversity of peptides in cone snail venom, a novel genetic and evolutionary strategy is employed by the genus as well.

Phylogenetic specificity of conopeptides

Pharmacological experiments carried out on crude venom by Endean and Rudkin (1963 and 1965) revealed that venom samples from *Conus* species that specifically prey on fish had no effect on mollusks or worms, and vice versa. These early studies suggested that the venom of a particular *Conus* species is presumably strongly selected to act on protein targets in the prey, and that the more unrelated the taxa to the prey, the more unlikely that it would have targets for the biologically active components in the venom.

Studies of individually isolated conopeptides have supported this general picture of the phylogenetic specificity of *Conus* venoms. A conopeptide from the venom of a particular species is generally specific for targets in the phylum to which the prey belongs. The conopeptides from the fish-hunting *Conus* venoms have been particularly well studied. Although in all cases specifically tested, the peptides have been inactive in invertebrate systems, they vary in how broadly they act. For example, α-conotoxins GI and MI from *Conus geographus* and *magus*, respectively, are broadly active in vertebrate systems, and appear to act at nicotinic acetylcholine receptors, not only in fish, but also in all mammalian systems tested. In contrast, α-conotoxin SI seems much more phylogenetically narrow in its biological activity: this peptide will inhibit nicotinic acetylcholine receptors in fish and in elasmobranchs (*Torpedo*), but its activity in mammalian systems is orders of magnitude less. Although the sequence changes are not very great, there are apparently strikingly different conformations assumed by these two peptides (Pardi *et al.*, 1990; Christensen *et al.*, 1991). The ω-conotoxins and μ-conotoxins, which target voltage-sensitive calcium channels and sodium channels, respectively, are also inactive in invertebrate systems. Considerable variation in the activity of the ω-conotoxins in different vertebrate systems has also been observed (see Ramilo *et al.*, 1992).

Thus, the general view of *Conus* venoms and conopeptides from studies on the fish-hunting species is that the agents to be found are likely to be vertebrate-specific. The studies on crude venoms suggest that worm-hunting species are likely to have conopeptides specific to annelids, and snail-hunting cones are likely to have conopeptides that are mollusk-specific. The one invertebrate-specific peptide tested so far, the King-Kong (KK) peptide from the snail-hunting species, *Conus textile*, is inactive in mammalian systems (Hillyard *et al.*, 1989).

However, peptides with broad phylogenetic specifity are desirable as tools for investigating the role of their receptor targets over a long period of evolutionary time. The present evidence from cloning suggests that most receptors and ion channels evolved early in eukaryotic evolution, and are widely used by all of the higher eukaryotic taxa. If a highly specific peptide ligand were able to inhibit the activity of such a receptor over a broad range of different phyla, the various uses to which that cell surface receptor had been put in the different taxa could be much more easily investigated. Thus, it is of interest to determine whether there are any conopeptides that have a broader specificity than those already characterized. In this article, we will show that a significant complement of conopeptides probably exhibit broad phylogenetic specificity.

Materials and Methods

Materials

Specimens of *Conus textile* were collected from the sea around the islands of Marinduque, Philippines. Venom

was extracted from the specimens as described previously (Cruz *et al.*, 1976).

Preparation of the venom extract

About one gram of the lyophilized venom was suspended in 10 ml of 1.1% acetic acid, placed on ice for 10 min with occasional stirring, then centrifuged at 12,000 $\times$ *g* in a Sorvall refrigerated centrifuge. The residue was extracted twice with 5 ml 1.1% acetic acid. Each time, the suspension was sonicated seven times at 65 watts (Sonifier, Heat Systems Ultrasonics, Inc.) for 10 s at 15-s intervals before centrifugation. The supernates were pooled and lyophilized.

Gel filtration chromatography

Lyophilized venom extract was dissolved in 5 ml of 1.1% acetic acid. The dissolved extract was chromatographed at 4°C on a column (110 $\times$ 2.5 cm) of Sephadex G-25 (fine), with 1.1% HAc as the eluent. The fractions corresponding to the chromatographic peaks were pooled, lyophilized, bioassayed, and stored at −20°C for further separation by HPLC.

High performance liquid chromatography

The lyophilized Sephadex G-25 fraction was redissolved in 2.0 ml of 0.1% trifluoroacetic acid (TFA), filtered, then chromatographed in several batches on a semi-preparative C18 column (TSK-ODS 120T, 7.8 $\times$ 300 mm, fully capped, 10 μm particle size). Subsequent fractionations were done on an analytical VYDAC reverse-phase C_{18} column (4.6 $\times$ 250 mm, 5 μm) and a Brownlee Spheri-5 ODS column (4.6 $\times$ 220 mm, 5 μm). The solvent system used for all HPLC runs consisted of 0.1% TFA as buffer A, and 0.1% TFA in 60% acetonitrile as buffer B.

Amino acid analysis

Peptide samples were hydrolyzed *in vacuo* with 6 *N* HCL/1% phenol for 18 h at 105°C. Amino acid analysis was done by reverse-phase HPLC of phenylthiocarbamyl derivatives (Bidlingmeyer *et al.*, 1984; Heinrikson and Meredith, 1984).

Peptide sequencing

The purified convulsant peptide was reduced and carboxymethylated as previously described (Cruz *et al.*, 1987) and then analyzed in a spinning-cup sequencer according to the method of Tarr *et al.* (1978). Phenylthiohydantoin derivatives were identified by HPLC using a gradient slightly modified from that of Hunkapiller and Hood (1978).

Results

Assay of a venom from a snail-hunting Conus *species for mammalian activity*

To examine whether components of a particular *Conus* venom are restricted in their activity to the phylum of its prey, we investigated the biological activities present in the venom of *Conus textile*. *Conus textile* is a common molluscivorous cone, and is one of the more highly successful large species in the genus. It is widely distributed from the Hawaiian Islands to the Red Sea, and is abundant in much of the Indo-Pacific. This species is of particular interest because it has been reported to be fatal to humans. However, when the venom was initially examined by Endean and Rudkin (1963), they did not find that it was active when tested in vertebrate systems.

We decided to re-examine the activity of *Conus textile* venom components for activity on mammalian receptors. Our laboratory has developed a sensitive assay for conopeptides that display activity in mammals. Direct injection of a venom component into the central nervous system of mice (i.c. injection) has proven to be an effective method for identifying vertebrate-active components (see Olivera *et al.*, 1990). The components of *Conus textile* venom were therefore assayed by i.c. injection into mice.

In this assay, both crude *Conus textile* venom, and all crude fractions assayed, had potent activity when injected intra-cranially into mice. Crude *Conus textile* venom causes seizure-like symptoms and death in mice. To determine the identity of a vertebrate-active component of the venom, we purified and characterized one venom component using biological activity in the mouse CNS as an assay.

Purification and characterization of a convulsant peptide from Conus textile *venom*

Conus textile venom was first fractionated according to size, as shown in Figure 1A. A major peptide fraction was then subfractionated by reverse-phase HPLC as shown in Figure 1B. The earliest eluting fraction from the first HPLC column caused paralysis and death in mice. This fraction was then rerun on reverse-phase HPLC. Two fractions in Figure 1C characteristically caused sudden jumping activity shortly after injection, and this was followed by convulsions, stretching of limbs, and jerking behavior. After these initial symptoms, the mouse would lie on its side and after approximately 25 min would tend to recover from the most severe symptoms. This general type of behavior was elicited by more than one peak in the chromatogram in Figure 1C, but only the indicated peak was further purified to homogeneity. Further HPLC purification finally yielded a homogeneous peptide with a convulsant activity (Fig. 1D). There may be, however, several isoforms of the convulsant peptide in *Conus textile* venom; this is being further investigated.

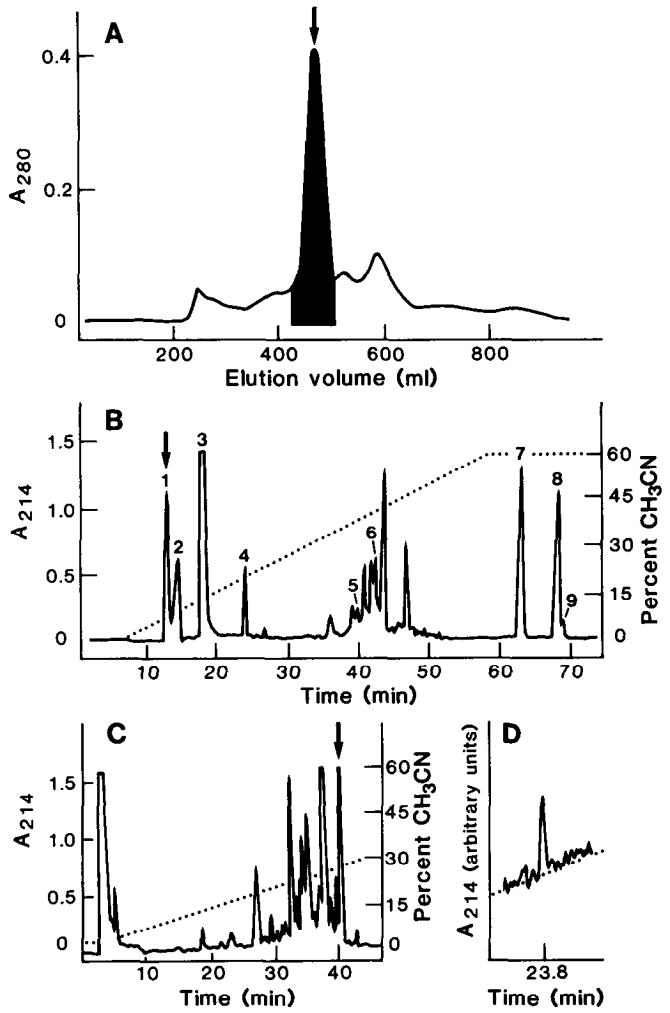

Figure 1. Purification of a convulsant peptide from *Conus textile* venom. Details of the procedure are described in Materials and Methods. (A) Column chromatography of crude venom extract on Sephadex G-25 with 1.1% acetic acid as eluent. (B) HPLC of pooled fractions from the shaded peak of Panel A on semi-preparative reversed phase C18 column at a gradient of 1.2% CH_3CN/min in 0.1% TFA. (C) The peak indicated by an arrow in B was further fractionated by HPLC on an analytical C18 column at 0.6% CH_3CN/min in 0.1% TFA. The peak corresponding to the convulsant peptide is indicated by an arrow. This was rerun on HPLC to remove impurities. (D) HPLC of purified convulsant peptide on a Brownlee C18 column using a 0.6% CH_3CN/min gradient in 0.1% TFA (23.8 min corresponds to 29.3% CH_3CN). The peaks indicated by numbers in panel B produced the following behavioral effects when injected intracranially in two-week old mice: (1) twitching, jerking, and paralysis; (2) jerking and stretching; (3) hyperactivity followed by weakness; (4) circular motion and jerking; (5) jumping, jerking, and running; (6) scratching and hyperactivity; (7) jumping and circular motion; (8) stretching; and (9) running and rolling over. Peaks 1, 3, 5, 6 and 9 are lethal.

The purified peptide was then subjected to amino acid analysis, as well as Edman sequencing. The results of these analyses are shown in Table I. The data are consistent with the convulsant peptide being a 23 amino acid peptide with the sequence:

NCPYCVVYCCPPAYCEASGCRPP.

To confirm the sequence and to determine whether the peptide was blocked at the carboxyl terminus, a fast atom bombardment (FAB) mass spectrometric analysis was carried out. The observed mass value of the convulsant peptide was 2487.90 mass units. The calculated value, if the peptide were amidated at the C-terminus, is 2487.95. These data therefore confirm the peptide sequence given

Table I

Determination of the primary structure of the convulsant peptide

A. Amino acid analysis

Amino acid*	pmol	Mole ratio**
Asp	57.9	0.7 (1)
Glu	149.9	1.9 (1)
Ser	87.1	1.1 (1)
Gly	107.3	1.3 (1)
Arg	83.5	1.0 (1)
Ala	137.7	1.7 (2)
Pro	390.6	4.9 (5)
Tyr	126.5	1.6 (3)
Val	71.9	0.9 (2)
Cys	153.5	1.9 (6)

B. Sequence analysis

Step	Assigned residues	Yield (pmol of PTH amino acid)
1	Asn	390
2	Cys*	400
3	Pro	270
4	Tyr	500
5	Cys*	320
6	Val	520
7	Val	560
8	Tyr	380
9	Cys*	300
10	Cys*	270
11	Pro	140
12	Pro	150
13	Ala	190
14	Tyr	240
15	Cys*	110
16	Glu	120
17	Ala	150
18	Ser	150
19	Gly	90
20	Cys*	77
21	Arg	30.5

Molecular weight: MH+ = 2487.90 Calc. MW = 2487.95

Sequence: NCPYCVVYCCPPAYCEASGCRPP*

* Cys residues were analyzed as the carboxymethyl adduct.

** Values in parenthesis indicate the number of amino acid residues found by sequence analysis as verified by FAB mass spectrometry.

The first step yielded a significant amount of glutamic acid as a contaminant. No residues were assigned for residues 22 and 23 since the yields for these steps were very low.

Table II

Major conopeptide structural frameworks

Four-loop conopeptides	(C---C---CC---C---C)
Example: ω-conotoxin GVIA	CKSPGSSCSPTSYNCCRSCNPYTKRCY*
Three-loop conopeptides	(CC---C---C---CC)
Example: μ-conotoxin GIIIA	RDCCTPPKKCKDRQCKPQRCCA*
Two-loop conopeptides	(CC---C---C)
Example: α-conotoxin GI	ECCNPACGRHYSC*

P = hydroxyproline; * denotes an amidated C-terminus.

above, and suggest that the convulsant peptide is amidated at the C-terminus, as is common among conopeptides.

Multiple peptides active in mammalian systems from Conus textile *venom*

During the purification of the convulsant peptide, we found that many other fractions exhibited activity in the mammalian central nervous system. Thus, the fractions from the first HPLC step were assayed for activity by the mouse i.c. injection assay. Many of the fractions (indicated by numbers in Fig. 1B) induce dramatic and diverse symptomatology in mice, and some fractions are lethal. Although the proportion of active fractions may be significantly lower than in a fish-hunting *Conus* venom, a very significant proportion of the total that were assayed still showed biological activity in the mammalian CNS.

These results indicate that many components of *Conus textile* venom have potent activity on receptors present in the mammalian central nervous system. Since the one component that was purified and characterized was a typical disulfide-rich conopeptide, we think it likely that *Conus* venoms will yield many such peptides with cross-phylum biological activity.

Discussion

The convulsant peptide described above has a number of interesting pharmacological and structural features. It defines a new subclass of conopeptides. As we have described previously, although the peptides in *Conus* venoms exhibit remarkable pharmacological diversity, the cysteine framework of these peptides is, in fact, highly conserved. Three major conopeptide classes of disulfide frameworks have been characterized so far, *i.e.*, the 2-loop, 3-loop, and 4-loop structural frameworks, as indicated in Table II (see Olivera *et al.*, 1990, 1991). The convulsant peptide clearly belongs to the class of conopeptides with four-loop structural frameworks.

But, within each structural framework class, rather distinctive subclasses emerge. For the four-loop conotoxin frameworks in particular, two distinctive subclasses were previously characterized; they are designated as Group I (the ω-conotoxin subclass) and Group II (the KK peptide subclass) in Table III. These subclasses are distinguishable by the overall biochemical characteristics of their peptides; in the case of the ω-conotoxins, the amino acids are overwhelmingly hydrophilic, and the peptides have a high net positive charge. In the case of the KK peptides, the intercysteine amino acids are considerably more hydrophobic, with a net negative charge.

The convulsant peptide differs from these two classes in having a very different number of amino acids present in each of the four amino acid loops between Cys residues. Between the first and second cysteine residues, and the second and third Cys, both the King-Kong peptides and the ω-conotoxins have 5–6 amino acids; in the convulsant peptide, the first two loops are extremely short with only two and three amino acids, respectively. A peptide with the same length distribution as the convulsant peptide has also been found in a worm-hunting cone, *Conus quercinus* (Abogadie *et al.*, 1990). The convulsant peptide and this latter conopeptide will probably define a new subclass within the four-loop structural framework class, distinct from either the KK peptide or the ω-conotoxin subclasses.

We are especially interested in determining the prepropeptide structure of the convulsant peptide. The prepro regions of the KK conopeptides and the ω-conotoxins have recently been found to be homologous but clearly divergent from each other (Colledge and Hillyard, 1992; D. Hillyard, unpub. results). In contrast, all KK peptides are highly homologous in the sequences of the prepro regions of the precursor molecule. If, as we postulate, the convulsant peptide defines a new subclass of four-loop conopeptides, we expect that the prepro sequences will exhibit some homology, but also considerable divergence from the KK and ω-conotoxin subclasses of precursors. It should then become possible to quickly identify other

Table III

Four-loop conopeptides; proposed subclasses

Name		Charge
Group I		
ω-Conotoxin GVIA	CKSPGSSCSPTSYNCCRSCNPYTKRCY*	+5
ω-Conotoxin MVIIA	CKGKGAKCSRLMYDCCTGSCRSGKC*	+6
Group II		
KK-0 peptide	WCKQSGEMCNLLDQNCCDGYCIVLVCT	−2
KK-1 peptide	CIEQFDPCEMIRHTCCVGVCFLMACI	−1.5
Group III		
Convulsant peptide	NCPYCVVYCCPPAYCEASGCRPP*	+1

The ω-conotoxins were described in Olivera *et al.*, 1985; the KK peptides in Woodward *et al.*, 1990. P stands for hydroxyproline; * means that the peptide is amidated at the C-terminus.

members of the convulsant peptide subclass by a molecular genetic approach; probes specific for the prepro regions should permit the facile identification of new conopeptides that are structurally within the convulsant peptide subclass.

The potent activity of this peptide in mammalian systems might not have been predicted from earlier data on crude venoms and well-characterized conopeptides from fish-hunting cone snails. Because the convulsant peptide is found in the venom of *Conus textile,* which clearly specializes in hunting other gastropod mollusks, finding a conopeptide that is highly potent in the mammalian central nervous system raises a number of interesting questions. Are there some biological reasons why *Conus textile* might have evolved a peptide that is active in mammalian systems? We think it more likely that the peptide has a receptor target that is highly conserved in both mammals and mollusks, and that this peptide will prove to have a particularly broad phylogenetic range.

The experiments described above provide a paradigm for quickly identifying a subset of peptides in a *Conus* venom that are likely to have a broad phylogenetic range, *i.e.,* assaying for biological activity in a phylum totally unrelated to the prey. Such peptides are probably not uncommon. In the preliminary survey of a *Conus textile* venom fraction shown in Figure 1B, we found a very significant proportion of all components in the venom active when injected into the central nervous system of mammals. The convulsant peptide is thus only the first of many such cross-phylum peptides to be characterized in detail.

It will be of interest to determine whether the subset of receptors targeted by such "broad-range" conopeptides is different from the subset of receptors targeted by narrow range conopeptides. However, further investigation into these issues will require the identification of the molecular targets of the convulsant peptide and other broad range conopeptides. We believe that the availability of "broad-range" conopeptides will be important in elucidating, not only the evolution of various ion channels and receptors, but ultimately the evolution of nervous systems in higher eukaryotes.

Acknowledgments

This work was supported by NIH grants GM 22737 (to BMO) and NS 27219 (to LJC), and by grants from the Marine Science Institute and the International Foundation for Science (Stockholm, Sweden). Amino acid analysis and sequencing were done in the laboratory of Dr. W. R. Gray, Dept. of Biology, University of Utah and mass spectrophotometry was done Dr. Terry Lee of the Beckman Research Institute of the City of Hope, California.

Literature Cited

Abogadie, F. C., C. A. Ramilo, G. P. Corpuz, and L. J. Cruz. 1990. Biologically active peptides from *Conus quercinus,* a worm-hunting species. *Trans. Natl. Acad. Sci. & Tech. (Philippines)* **12:** 219–232.

Bidlingmeyer, B. A., S. A. Cohen, and T. L. Tarvin. 1984. Rapid analysis of amino acids using pre-column derivatization. *J. Chromatogr.* **336:** 93–104.

Christensen, D. J., C. D. Poulter, R. A. Myers, and B. M. Olivera. 1992. Determination of the solution conformation of conotoxin SI by two-dimensional NMR. *Abstracts of Papers, 203rd National Meeting of the American Chemical Society.* Abstr. No. 331.

Colledge, C. J., J. P. Hunsperger, J. S. Imperial, and D. R. Hillyard. 1992. Precursor structure of ω-conotoxin GVIA determined from a cDNA clone. *Toxicon* (in press).

Cruz, L. J., G. Corpuz, and B. M. Olivera. 1976. A preliminary study of *Conus* venom protein. *Veliger* **18:** 302–308.

Cruz, L. J., V. de Santos, G. C. Zafaralla, C. A. Ramilo, R. Zeikus, W. R. Gray, and B. M. Olivera. 1987. Invertebrate vasopressin/oxytocin homologs. Characterization of peptides from *Conus geographus* and *Conus striatus* venoms. *J. Biol. Chem.* **262:** 15821–15824.

Endean, R., and C. Rudkin. 1963. Studies of the venoms of some Conidae. *Toxicon* **1:** 49–64.

Endean, R., and C. Rudkin. 1965. Further studies of the venoms of Conidae. *Toxicon* **2:** 225–249.

Heinrikson, R. L., and S. C. Meredith. 1984. Amino acid analysis by reverse-phase high-performance liquid chromatography: precolumn derivatization with phenylisothiocyanate. *Anal. Biochem.* **136:** 65–74.

Hunkapiller, M. W., and L. E. Hood. 1978. Direct microsequence analyses of polypeptides using an improved sequenator, a nonprotein carrier (polybrene) and high pressure liquid chromatography. *Biochemistry* **17:** 2124–2133.

Olivera, B. M., W. R. Gray, R. Zeikus, J. M. McIntosh, J. Varga, J. Rivier, V. de Santos, and L. J. Cruz. 1985. Peptide neurotoxins from fish-hunting cone snails. *Science* **230:** 1338–1343.

Olivera, B. M., J. Rivier, C. Clark, C. A. Ramilo, G. P. Corpuz, F. C. Abogadie, E. E. Mena, S. R. Woodward, D. R. Hillyard, and L. J. Cruz. 1990. Diversity of *Conus* neuropeptides. *Science* **249:** 257–263.

Olivera, B. M., J. Rivier, J. K. Scott, D. R. Hillyard, and L. J. Cruz. 1991. Conotoxins. *J. Biol. Chem.* **33:** 22067–22070.

Pardi, A., A. Goldes, J. Florance, and D. Maniconte. 1989. Solution structures of α-conotoxins determined by two-dimensional NMR spectroscopy. *Biochemistry* **28:** 5494–5501.

Ramilo, C. A., G. C. Zafaralla, L. Nadasdi, L. G. Hammerland, D. Yoshikami, W. R. Gray, J. Ramachandran, G. Miljanich, B. M. Olivera, and L. J. Cruz. 1992. Novel α- and ω-conotoxins from *Conus striatus* venom. *Biochemistry* (in press).

Tarr, G. E., J. F. Beecher, M. Bell, and D. J. McKean. 1978. Polyquarternary amines prevent peptide loss from sequenators. *Anal. Biochem.* **84:** 622–627.

Woodward, S. R., L. J. Cruz, B. M. Olivera, and D. R. Hillyard. 1990. Constant and hypervariable regions in conotoxin polypeptides. *EMBO J.* **9:** 1015–1020.