Conotoxins are multiple disulfide-bonded peptides isolated from marine cone snail venom. These toxins have been classified into several families based on their disulfide pattern and biological properties. Here, we report a new family of Conus peptides, which have a novel cysteine motif. Three peptides of this family (CMrVIA, CMrVIB, and CMrX) have been purified from Conus marmoreus venom, and their structures have been determined. Their amino acid sequences are VCCGYKLCIOC (CMrVIA), NVGCCGYKLCIOC (CMrVIB), and GICC GVFCYCOC (CMrX), where O represents 4-trans-hydroxyproline. Two of these peptides (CMrVIA and CMrX) have been chemically synthesized. Using a selective protection and deprotection strategy during disulfide bond formation, peptides with both feasible cysteine-pairing combinations were generated. The disulfide pattern (C1-C4, C2-C3) in native toxins was identified by their co-elution with the synthetic disulfide-isomeric peptides on reverse-phase high pressure liquid chromatography. Although cysteine residues were found in comparable positions with those of α-conotoxins, these toxins exhibited a distinctly different disulfide bonding pattern; we have named this new family “λ-conotoxins.” CMrVIA and CMrX induced different biological effects when injected intra-cerebroventricularly in mice; CMrVIA induces seizures, whereas CMrX induces flaccid paralysis. The synthetic peptide with λ-conotoxin folding is about 1150-fold more potent in inducing seizures than the mispaired isomer with α-conotoxin folding. Thus it appears that the unique disulfide pattern, and hence the “ribbon” conformation, in λ-conotoxins is important for their biological activity.

Conotoxins are biologically active peptide toxins isolated from venomous marine cone snails. They are typically small disulfide-rich peptides containing 11–30 amino acid residues. Conotoxins can be classified into several families based on the number and pattern of disulfide bonds and biological activities (1, 2). Members of a single family of conotoxins share similar protein folding but in some cases exhibit different biological activities (3–5). These differences in biological activities are due to their ability to bind with specific ion channels or receptors (6). Some of these conotoxins are used as tools in investigating receptor structure and function (2) and ion channel geometry (7). The structurally constrained scaffolds of conotoxins are utilized as a template for protein engineering to create chimeric proteins (8, 9).

Conotoxins, like toxins from other venoms, evolve rapidly by positive Darwinian selection (10). The specificity of conotoxins is due to their disulfide bonding framework and specific amino acids in inter-cysteine loops. The high density of disulfides in conotoxins plays a vital role in their stability and imposes a distinct protein folding with a specific orientation of hypervariable loop regions. The diversity in conotoxins is also achieved through disulfide pairings (see “Discussion”).

The conotoxins with two disulfide bonds can have three possible monomeric conformations: “globular” having a Greek φ-like conformation formed by interlocking disulfide bridges between C1-C3 and C2-C4, “string of independent bead” conformation with C1-C2 and C3-C4, or a “ribbon topology” with C1-C4 and C2-C3 bond formation (11). Native α- and r-conotoxins, however, possess an identical disulfide bonding pattern (C1-C3, C2-C4) and globular conformation (12, 13). All three disulfide isomers of α-conotoxin (GI from Conus geographus venom) have been synthesized, and the native structure corresponded to the most stable isomer with C1-C3 and C2-C4 pairing (14, 15). Two other isomers are relatively unstable. In addition, the C1-C4, C2-C4 isomer rearranges more readily than does the C1-C2, C3-C4 isomer to the native C1-C3, C2-C4 isomer in various buffers containing shuffling reagent (15). Furthermore, the isomer with C1-C3 and C2-C4 disulfide pairing is at least 10 times more potent than the other disulfide isomers (14, 15). Collectively, these studies indicate that the C1-C4, C2-C3 isomer is the least active and unstable among two-disulfide conotoxins.

In this study, we report the members of a novel family of conotoxins, which we have named “λ-conotoxins.” Their unique disulfide pattern (C1-C4, C2-C3) leads to a new native protein fold with ribbon topology in conotoxins, exposing different pep-
Here we describe the purification and characterization of three conotoxins of this new family. Their disulfide bond pattern was established by specific chemical synthesis and co-elution. The synthetic peptides with native conformation in this family exhibit different biological activities. The critical nature of the ribbon topology in native \(\lambda\)-conotoxins is substantiated by their potent biological activity.

**EXPERIMENTAL PROCEDURES**

Materials—Reverse-phase HPLC: Jupiter C18 (4.6 mm \(\times\) 250 mm) and Sephasil C18 (2.1 mm \(\times\) 100 mm) columns were obtained from Phenomenex (Torrance, CA) and Amersham Pharmacia Biotech, respectively. Reagents for amino-terminal sequencing were from Applied Biosystems (Foster City, CA). Acetonitrile was purchased from Fisher, and 4-vinylpyridine and trifluoroacetic acid were purchased from Fluka Chemika-Biochemika (Buchs, Switzerland). Sterile, non-pyrogen 0.9% NaCl injection British Pharmacopea was from B/Braun, Melsungen AG (Penang, Malaysia). Fmoc-amino acids, Fmoc-Cys(trityl)-resin, and other synthesis reagents were obtained from PerkinElmer Life Sciences, Watanabe Chemical Industries Ltd. (Hiroshima, Japan), Peptide Institute (Osaka, Japan), and Kokusan Chemical Works Ltd. (Tokyo, Japan). All other chemicals used were of analytical grade.

Peptide Purification by Reverse-phase Chromatography—The venom of *Conus marmoreus* was extracted from the venom duct of specimens collected around Singapore waters (16). It was loaded on a reverse-phase HPLC Jupiter C18 column (4.6 mm \(\times\) 250 mm) run on a Vision Workstation (PerkinElmer Life Sciences). The peptide peaks were re-loaded onto a Sephasil C18 column (2.1 mm \(\times\) 100 mm) on a Smart System (Amersham Pharmacia Biotech). Both columns were equilibrated with 0.1% trifluoroacetic acid (solvent A), and bound peptides were eluted using a linear gradient of 80% acetonitrile in 0.1% trifluoroacetic acid (solvent B).

Electrospray Mass Spectrometry—The molecular masses of the peptides were determined by electrospray ionization mass spectrometry on a PerkinElmer ScieX API III triple-stage quadrupole instrument equipped with an ionspray interface (ScieX, Thornhill, Canada) essentially as described earlier (17).

Reduction, Alkylation, and Sequencing of Peptides—The peptides were dissolved in 100 \(\mu\)l of the denaturation buffer (6.0 M guanidinium hydrochloride, 0.13 M Tris, 1 mM EDTA, pH 8.0) containing 0.07 M \(\beta\)-mercaptoethanol. The mixture was incubated under nitrogen at 37 °C for 2 h. Subsequently, a 1.5-fold molar excess (over sulfhydryl groups) of 4-vinylpyridine was added and incubated under nitrogen at room temperature. After 2 h, samples were desalted on a Sephasil C18 column (2.1 mm \(\times\) 100 mm). Amino-terminal sequencing of the native and pyridylethylated peptides was performed by automated Edman degra-

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2 The abbreviations used are: HPLC, high pressure liquid chromatography; Fmoc, \(N\)-(9-fluorenyl)methoxycarbonyl; PTH, phenylthiohydantoin; ODS, octadeceansilane.
dation using a PerkinElmer Life Sciences 494 pulsed-liquid phase protein sequencer (Procise). Phenylthiohydantoin amino acids were identified using an on-line reverse-phase HPLC (PTH-C18 column) with an on-line 785A PTH-amino acid 140C analyzer. The modified amino acid, 4-trans-hydroxyproline, in the peptide was confirmed by comparing the elution position of free 4-trans-hydroxyproline in the amino acid analysis with that found in the normal Edman sequencing of the native and pyridylethylated peptide.

Synthesis and Purification of Peptides—Solid phase peptide synthesis was performed on a PerkinElmer Life Sciences 433A peptide synthesizer. Amino acid analyses were performed on a Beckman System Gold amino acid analyzer after hydrolysis in 6 M HCl at 110 °C for 24 h and derivatization by 4-dimethylaminoazobenzene-4'-sulfonyl chloride. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry was carried out with a PerSeptive Biosystems Voyager Linear DE mass spectrometer using a-cyano-4-hydroxy-cinnamic acid as a matrix. Analytical and preparative HPLC were conducted on a Shimadzu LC-10A system with the ODS columns Shim-pack CLC-ODS (4.6 × 250 mm, Shimadzu) and Shim-pack PREP-ODS (H) (20 × 250 mm, Shimadzu), respectively.

For the synthesis of isomer A of CMrVIA, a linear peptide containing Cys(acetamidomethyl) at the 2nd and 8th positions was assembled on the resin by solid phase methodology of Fmoc chemistry in a 0.25-mmol scale. We used a selective protection and deprotection strategy during disulfide bond formation to generate two different disulfide isomers (Fig. 1). After trifluoroacetic acid cleavage of a 0.15-mmol equivalent of the peptide resin, a linear peptide with free Cys residues at the 3rd and 11th positions was extracted with 2 M AcOH (375 ml), and the solution was adjusted to pH 7.8 with aqueous NH₄OH and diluted to 1500 ml. The final concentration was 0.1 m for peptide and 0.05 m for ammonium acetate buffer. The solution was stirred slowly at room temperature to form the first disulfide bond. The monocyclic peptide was purified by gel filtration and preparative HPLC (yield, 138 mg; 66% from starting resin). The monocyclic peptide (17 mg, 0.012 mmol) dissolved in 80% aqueous methanol (24 ml) was dropped into a solution of iodine (123 mg) in the mixture (24 ml) of methanol, 1 M HCl (4:1) to form a second disulfide bond between C2 and C8. The bicyclic peptide was purified by preparative HPLC (yield: 6.9 mg, 29%). The structure and purity of synthetic peptides were confirmed by analytical HPLC, amino acid analysis, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry measurements. Isomer B of CMrVIA was essentially synthesized as described for isomer A but using Cys(acetamidomethyl) at the 3rd and 8th positions. The yield from starting resin was 47% for the monocyclic peptide and 9% for the final product.

Isomers A and B of CMrX were synthesized by the same strategy as described for CMrVIA isomers. In the case of isomer A, the yield from starting resin was 28% for the monocyclic peptide and 5% for the final product. In the case of isomer B, the yield was 52 and 8%, respectively.

### TABLE I

| Conotoxin | Peptide mass (masscal) | Observed mass (massobs) | Pyridylethylated peptide mass (masscal) | Observed mass (massobs) |
|-----------|------------------------|-------------------------|----------------------------------------|-------------------------|
| CMrVIA    | 1237.56                | 1237.93 ± 0.21          | 1661.96                                | 1661.47 ± 0.30          |
| CMrVIB    | 1408.65                | 1408.97 ± 0.09          | 1833.06                                | 1833.02 ± 0.86          |
| CMrX      | 1263.49                | 1262.77 ± 0.07          | 1687.89                                | 1687.17 ± 0.44          |

### FIG. 3. Purity and co-elution of synthetic isomers (isomer A and isomer B) of CMrVIA and CMrX. A, HPLC profiles of native and synthetic CMrVIA isomers. Column, Shim-pack CLC-ODS (4.6 mm × 250 mm, Shimadzu); solvent, 20% CH₃CN in aqueous 0.1% trifluoroacetic acid; flow rate, 1 ml/min; monitoring, absorbance at 230 nm (intensity not scaled). B, HPLC profiles of native and synthetic CMrX isomers. Column, Shim-pack CLC-ODS (4.6 × 250 mm, Shimadzu); solvent, 25% CH₃CN in aqueous 0.1% trifluoroacetic acid; flow rate, 1 ml/min; monitoring, absorbance at 230 nm (intensity not scaled).
**Biological Activity**—The biological activities of the synthetic peptides were determined by intracerebroventricular injections in young Swiss mice (10–15 g). A volume of 10 μl of conotoxin in normal saline was injected using a fine capillary Hamilton microliter syringe into mice under ether anesthesia, and the same volume of normal saline was injected into control animals. Clonic seizures were defined as continuous limb clonus lasting 3 s or longer in duration; and when the hind limbs were fully extended in the plane of the body, it was considered a tonic seizure. The mice were sacrificed with ether anesthesia and cervical dislocation immediately after the observation period. Percentage responses represent the fraction of mice showing seizures. The test was carried out in four to six groups of at least three animals in each group. ED₅₀ values are the dose at which 50% of tested animals showed seizure determined by Probit analysis.

**Molecular Modeling**—The structure of ribbon CMrVIA was modeled based on the coordinates of the ribbon GI (C¹-C³, C²-C⁴) isomer (Brookhaven Protein Data Bank accession code 1XGB) structure determined by Gehrmann et al. (15). To generate the backbone coordinates of CMrVIA, its sequence was manually aligned with that of GI followed by backbone residue replacement of GI with the corresponding residues from CMrVIA. The generated structure was modified with an insertion of a trans-4-hydroxyl group to the proline residue. This structure was then thoroughly minimized with all force constants at full value and the Lennard-Jones form of nonbond force field using both steepest decent and conjugate gradient minimizations.

**RESULTS**

**Purification of Conotoxins**—Conotoxins isolated from C. marmoratus venom by reverse-phase-HPLC are shown in Fig. 2A. All A₂₁₅-absorbing peaks were analyzed by electro spray ionization mass spectrometry to determine their purities and molecular masses. More than 15 peptides were purified to homogeneity. These conotoxins were named CMr peptides to avoid potential confusion with the MrI-MrXV peptides purified from C. marmoratus venom by Fainzilber et al. (18). CMrVIA, CMrVIB, and CMrX eluted between a 40–60% gradient of Solvent B. CMrVIA and CMrVIB peptides were further separated using a slower gradient on a Sephasil C18 column (Fig. 2). The homogeneity of the native CMrVIA, CMrVIB, and CMrX peptides was determined by electrospray ionization mass spectrometry, and the masses were found to be 1237.93, 1408.97, and 1262.77 Da, respectively (Fig. 2, C–E).

**Determination of Amino Acid Sequence**—The native peptides were sequenced end to end by automated Edman degradation. CMrVIA, CMrVIB, and CMrX possess 11, 13, and 12 amino acids, respectively. The presence of cysteines was confirmed by sequencing reduced and pyridylethylated conotoxins. The penultimate position of these toxins contained an uncommon amino acid residue. The PTH-amino acid in these cycles showed two peaks; the first, shorter peak eluted immediately after PTH-His, and the second, taller peak eluted after PTH-Ala (data not shown). Based on the calculated mass of the peptide and the observed mass, the mass of the unknown residue (X) was 113.10. This corresponds to the mass of leucine, isoleucine, or 4-hydroxyproline residues. Because the mass of the PTH derivative did not correspond to the mass of leucine or isoleucine, we loaded free 4-trans-hydroxyproline onto the sequencer. The PTH derivative(s) of 4-trans-hydroxyproline eluted as two peaks at positions similar to those observed for the penultimate residue, confirming that it was 4-trans-hydroxyproline. The amino acid sequences are VCCGYKLCHOC (CMrVIA), NGVCCGYKLCHOC (CMrVIB), and GICGVSFCYOC (CMrX), where O is 4-trans-hydroxyproline. The calculated molecular mass of the conotoxins matched the observed molecular mass, indicating the presence of a free carboxyl terminus (Table I). CMrVIB has an additional two residues at the amino-terminal end of CMrVIA. A similar toxin pair has been isolated from fish hunting cone snail Conus consors (CnIA and CnIB) (19). A Basic Local Alignment Search Tool search (20) of the data base did not show any significant homology to any of the known proteins or peptides, indicating that these are structurally novel conotoxins.

**Peptide Synthesis and Assignment of Disulfide Pattern**—Two of the three conotoxins were synthesized to provide sufficient material for further study. Because all α-conotoxins have C¹-C³, C²-C⁴ pairing, CMrVIA isomer A was synthesized first with this disulfide pairing (Fig. 1). To our surprise isomer A did not have retention characteristics similar to those of the native...
conotoxin (Fig. 3A). Therefore, we synthesized CMrVIA isomer B, which co-eluted with the native peptide (Fig. 3A). We did not synthesize isoconopeptide C with C1-C3, C3-C4 pairing, because vicinyl disulfide bonds are extremely rare. Isomers A and B of CMrX were synthesized in small quantities to check for its natural folding. It also has C1-C4, C2-C3 pairing (Fig. 3B). All bicyclic peptides were 97–98% pure with the correct monoisotopic mass (data not shown). The synthesis validated the free carboxyl terminus and the presence of 4-trans-hydroxyproline. The synthesis of CMrVIB was unsuccessful because of side reactions, such as cyclization and isomerization of the Asn-Gly sequence. The co-elution of isoconopeptides B with the native peptides (Fig. 3) indicates that the native conotoxins have C1-C4, C2-C3 disulfide linkages. We designated this new class of conotoxins “λ-conotoxins.”

Molecular Modeling of λ-Conotoxins—The three synthetic disulfide isoforms of α-conotoxin GI show three significantly different structures (15). Except for the native conotoxin-GI (globular form), the two non-native isoforms (ribbon and string of bead forms) exhibit multiple conformations. The globular structure has a central region of α-helix and a series of overlapping β-turns at the amino and carboxyl termini, whereas the ribbon structure has a β-bulge between C2 and C3 with nested β-turns (15, Fig. 4B), and the string of bead structure has an ordered amino terminus and a disordered carboxyl terminus (data not shown). Using the ribbon conformation of conotoxin GI as a template, the CMrVIA structure was modeled to understand the structural details (Fig. 4A). The Ramachandran plot was used to judge the quality of the CMrVIA model. All of the φ and ψ dihedral angles (with the exception of glycine) fall within the permissible regions according to the plot (data not shown). This demonstrates that the rigorous energy minimization algorithms employed successfully resolved both torsion angle and steric contact issues. CMrVIA has two loops consisting of residues 4–7, 9, and 10. The sizes of these loops are conserved in all three λ-conotoxins. The second loop is significantly smaller than that of α-conotoxins. The presence of a conserved hydroxyproline residue within the loop is likely to impose rigidity upon the second loop. The longer, second loop in α-conotoxins is relatively more flexible (15). The backbone in λ-conotoxins is more likely to be flat (ribbon conformation) with the amino and carboxyl termini proximate due to tethering by the C1-C4 disulfide bond (Fig. 4B).

Biological Activities of λ-Conotoxins—Administration of CMrVIA in mice by intracerebroventricular injection (20 ng/g of body weight) induced complex seizures. The time-dependent behavioral changes induced by CMrVIA isoconopeptide are shown in Table II. Although both isoconopeptides induced similar symptoms, isoconopeptide A was relatively less potent. The symptoms were dose-dependent; with increasing doses, symptoms were more severe, and the time lags were shorter. At 40 ng/g of body weight (isoconopeptide B), mice died within 20–30 s. In the case of isoconopeptide A, the first symptoms of clonic seizures were observed after a lag period of 7–10 min of toxin administration compared with isoconopeptide B (0.5–5 min). We determined the dose response for both isoconopeptides in inducing seizures (Fig. 5). Based on ED50 values (the dose required to induce seizures in 50% of mice), native CMrVIA isoconopeptide B (λ-conotoxin folding) was three orders of magnitude more potent than isoconopeptide A (α-conotoxin folding). Thus the novel disulfide bonding pattern and protein fold of λ-conotoxins appear to be physiologically relevant. In contrast, GI with native α-conotoxin folding was 10-fold more potent than the synthetic non-native isoconopeptide with λ-conotoxin folding (14, 15).

Unlike CMrVIA, CMrX isoconopeptide B (12.6 μg/g of body weight). The time-dependent series of symptoms observed in mice is shown in Table II. α-Conotoxins induce similar symptoms (21). The studies on the relative potency of isoconopeptide A and isoconopeptide B of CMrX could not be carried out, because we synthesized these isoforms in small quantities only to confirm the disulfide pairing pattern.

Because λ-conotoxins interfered with neurotransmission, we examined the effect of CMrVIA and CMrX on chich biventercervisi precapillarization. Both peptides showed negligible effects on neuromuscular transmission at concentrations up to 124 and 126 nM, respectively.

DISCUSSION

The venoms of Conus snails are a complex mixture of pharmacologically active peptides. These disulfide-rich, compact peptides called conotoxins are classified into several different families based on their structure and biological activity (Fig. 6). Structurally, the disulfide bonds are intrinsic to the stable

Table II

| Time (min) | Behavioral symptoms |
|-----------|---------------------|
| 1–10      | Induced by CMrVIA   |
|           | Hyperactivity, head tremors, wet dog shakes, biting, intermittently paroxysmal (2–5 s) of unilateral forelimb and hind limb clonus with urination |
| 2–60      | Confused wild running and jumping, barrel rolling, rearing and falling, spinning, stiff tail |
| 10–120    | Tongue biting, squeaking, tonic hind limb extension, urination and death |
| 1–15      | Induced by CMrX     |
|           | Flaccid paralysis of limbs, unable to move even after tail pinching, eyes open |
| 15–120    | Breathing difficulty, rapid movement of nostrils, death |

Fig. 5. Dose-response relationship for seizure induction by CMrVIA isoconopeptide A and CMrVIA isoconopeptide B upon intracerebroventricular administration in mice.
three-dimensional conformation and play an important role in their function. Hence, a change in the disulfide bonding pattern leads to a significant change in the overall conformation and function of the toxin. For example, in the case of conotoxins with three-disulfide bonds at least five structural families (or templates) with at least two distinct disulfide pairings have been documented (Fig. 6). These structural changes contribute to the difference in their folding and biological functions (for review see Ref. 1).

So far two families of toxins (α and τ) have been identified in conotoxins with two-disulfide bonds (Fig. 6). They share a similar disulfide pairing, i.e. C1-C3 and C2-C4, and have a globular structure. The alternative disulfide bonding pattern and protein folds are considered unstable and physiologically irrelevant (15).

Here, we report the isolation and characterization of the first members of a new family of conotoxins, which we have named λ-conotoxins, with a different disulfide pairing (C1-C4 and C2-C3), hitherto not reported in native conotoxins.

λ-conotoxins share some features with α-conotoxins: (a) they are smaller in size and have 11–16 amino acid residues (Fig. 6), (b) they have four cysteines in comparable positions, and (c) they have two disulfide bonds forming two loops. However, they have several characteristic features that differ from α-conotoxins. First, λ-conotoxins differ from α-conotoxins in their disulfide bonding patterns. α-Conotoxins have C1-C3 and C2-C4 pairing, whereas λ-conotoxins have C1-C4 and C2-C3 pairing (Fig. 4). Second, they differ in their loop sizes. The second loop in α-conotoxins is larger than the first loop (except for ImI); in contrast, the second loop is smaller in λ-conotoxins (Fig. 4A). Third, they differ in the positions of conformation-constraining residues. α-Conotoxins have a proline in the first loop, whereas λ-conotoxins have a hydroxyproline in the second loop. In α-conotoxins the second loop is more flexible, whereas in λ-conotoxins the first loop is more flexible. Fourth, they differ in carboxyl-terminal amidation. Carboxyl-terminal residues of all but one α-conotoxin (AuIC) are amidated; on the other hand,
carboxyl-terminal residues of \(\alpha\)-conotoxins are not amidated. Fifth, they differ in their overall conformation. \(\alpha\)-Conotoxins have a globular conformation that is crucial for proper molecular recognition (15, Fig. 4B). In contrast, \(\lambda\)-conotoxins have a flat ribbon-like conformation (Fig. 4B). This native conformation appears to be important for the biological activity of \(\lambda\)-conotoxins. CMrVIA with the native conformation (isomer B) is about 1150-fold more potent in inducing seizures than the one with \(\alpha\)-conotoxin fold (isomer A) (Fig. 5). This alternative cystine pairing and ribbon conformation found in natural toxins may be useful as a template in developing a new family of lead compounds in the drug discovery process.

Conus venoms are a natural pharmacopoeia of toxins that can bind to and discriminate closely related receptors and ion channels. They have evolved as a diverse group of toxin peptides in several ways. First, they have evolved by varying their loop structures. Like any other key physiological proteins, the loop regions within conotoxins are essential for biological specificity. The divergence is achieved through a change in loop size, number of loops/interacting surfaces, and loop composition through hyper-mutation. Second, they have evolved by adopting post-translational modifications. Several post-translational modifications occur in conotoxins, which also increase the complexity. These modifications help either in protein-protein interaction or in modifying the physiochemical properties (1). Third, they have evolved by differential disulfide formation. In conotoxins with three disulfides, different disulfide pairings alter the protein folding, conformation, and function. As shown here, the two-disulfide conotoxins also have alternate disulfide pairing. In both \(\alpha\) and \(\lambda\)-conotoxins, the native cysteine pairing, and hence the conformation, is critical for their biological activity. Thus, alternative disulfide pairings also contribute to structural and functional diversity in conotoxins.

In summary, \(\lambda\)-conotoxins are a novel family of conotoxins differing from \(\alpha\)-conotoxins in several structural and functional characteristics. We have isolated and characterized three \(\lambda\)-conotoxins from \(C. marmoratus\) venom. Structural studies of this family could provide better insight into the usefulness of this new scaffold in molecular engineering, the development of peptide libraries, and the design of potential drug prototypes.

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