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Neurotoxins from the venoms of the sea snakes *Hydrophis ornatus* and *Hydrophis lapemoides*

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The main neurotoxic components, toxins Hydrophis ornatus a and Hydrophis lapemoides a, were isolated from the venoms of the sea snakes *Hydrophis ornatus* and *Hydrophis lapemoides* respectively. The amino acid sequence of toxin Hydrophis ornatus a was deduced to be identical with that of toxin Astrotia stokesii a [Maeda & Tamiya (1978) *Biochem. J.* **175**, 507–517] on the basis of identity of the tryptic peptide 'map' and the amino acid composition of each peptide. The amino acid sequence of toxin Hydrophis lapemoides a was determined mainly on the basis of identity of the amino acid compositions, mobilities on paper electrophoresis and migration positions on paper chromatography of the tryptic peptides with those of other sea-snake toxins whose sequences are known. Both toxins Hydrophis ornatus a and Hydrophis lapemoides a consisted of 60 amino acid residues and there were six amino acid replacements between them. The taxonomy of sea snakes in the *Hydrophis ornatus* complex has long been confused, and the above snakes were originally assigned to taxa that proved to be inconsistent with the relationships indicated by the neurotoxin amino acid sequences obtained. A subsequent re-examination of the specimens revealed an error in the original identifications and demonstrated the value of the protein amino acid sequences in systematic and phylogenetic studies. The isolation procedure and results of amino acid analysis of the tryptic peptides have been deposited as Supplementary Publication SUP 50121 (8 pages) with the British Library Lending Division, Boston Spa, Wetherby, West Yorkshire LS23 7BQ, U.K., from whom copies may be obtained as indicated in *Biochem. J.* (1983) **209**, 5.

During the period December 1972–January 1973, in the course of an expedition to Ashmore Reef in the Timor Sea by the Research Vessel *Alpha Helix* of the Scripps Institution of Oceanography, a number of specimens of the hydrophiid sea snake *Hydrophis ornatus* (Gray 1842) were taken in McCluer Gulf, Irian Jaya. On a second *Alpha Helix* expedition to the Philippine Islands in August–September 1975, numerous specimens of two similar *Hydrophis* were collected and, on the basis of external morphology, were assigned to *Hydrophis ornatus* (*Hydrophis lapemoides* in the present paper) and *Hydrophus inornatus* (Gray 1849; *Hydrophis ornatus* in the present paper) (Minton & Dunson, 1978). Venom was milked from these live snakes on the *Alpha Helix*, freeze-dried and brought back to the laboratory. The present paper describes the isolation of a major neurotoxin from each species and the amino acid sequences deduced on homology to the neurotoxins of known sequences.

When it became evident that the identifications based on morphological criteria were inconsistent with the taxonomic relationship indicated by the toxin amino acid sequences, the specimens were re-examined and some of the original identifications were found to be in error. Although the taxonomy of this group of sea snakes continues to be confused because of inadequate sampling, the value of the amino acid sequences of their neurotoxin molecules in elucidating taxonomic relationships is clearly demonstrated.

Experimental and results

All the methods used in venom and venom component analyses were the same as those described previously (Maeda & Tamiya, 1976).

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Chromatography of the venoms

The crude venom (54.1 mg dry wt. from 0.155 ml of wet venom) of *Hydrophis ornatus* from McCluer Gulf was dissolved in 0.01 M phosphate buffer (Na_2HPO_4/KH_2PO_4), pH 6.4 (10 ml), and applied to a CM-cellulose (CM-52; Whatman, Maidstone, Kent, U.K.) column (1.5 cm × 19 cm) equilibrated with the same buffer. The elution pattern is given in Fig. 1. The fraction with lethal activity to mice was collected and freeze-dried, and the freeze-dried material was dissolved in 0.1 M-acetic acid (2 ml) and applied to a Sephadex G-50 (fine grade) column (1.6 cm × 100 cm) in 0.1 M-acetic acid. Toxin Hydrophis ornatus a (from the McCluer Gulf venom preparation), obtained in a pure state, accounted for 42% of protein and 70% of toxicity of the whole venom.

The crude venom (30.6 mg dry wt. from 0.19 ml of wet venom) of *Hydrophis ornatus* from the Philippines was dissolved in 0.01 M-phosphate buffer, pH 6.4 (10 ml), and applied to a CM-cellulose (CM-52) column (1.5 × 17 cm) and chromatographed (Fig. 2). The elution pattern was very similar to that of *Hydrophis ornatus* venom from the McCluer Gulf venom preparation. The component in the fractions indicated by the horizontal bar was pure on disc-gel electrophoresis at pH 4.0, and named toxin Hydrophis ornatus a (from the Philippines venom preparation). The recovery of the protein in this fraction was 58%.

The crude venom (37 mg) of *Hydrophis lapemoides* from the Philippines was chromatographed in the same way as that of *Hydrophis ornatus* venom from the Philippines. The elution pattern (Fig. 3) was quite different from those of the venoms of *Hydrophis ornatus* from McCluer Gulf or the Philippines. The main toxic component was eluted at higher NaCl concentration (0.16 M) than was toxin Hydrophis ornatus a (0.12 M). This fraction was pure on disc-gel electrophoresis at pH 4.0 and named toxin Hydrophis lapemoides a. The component accounted for 44% of the protein of the whole venom. The LD_{50} value of the toxin by intramuscular injection to mice (body wt. 20 g) was 0.09 μg/g body wt.

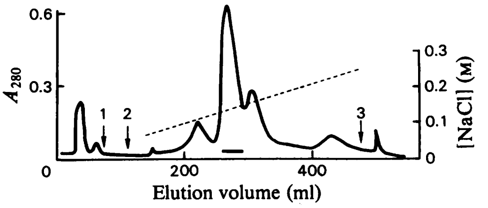

Fig. 1. *CM-cellulose (CM-52) column chromatography of Hydrophis ornatus venom preparation from the McCluer Gulf*

Freeze-dried venom preparation (54.1 mg) was dissolved in 0.01 M-phosphate buffer, pH 6.4 (10 ml), applied to a CM-cellulose (CM-52) column (1.5 cm × 19 cm) and chromatographed with the same buffer. At arrow 1, the buffer was changed to that containing 0.05 M-NaCl, and at arrow 2, a linear gradient in NaCl concentration was applied with 200 ml of the buffer containing 0.05 M-NaCl in the mixing chamber and the buffer containing 0.3 M-NaCl in the reservoir (200 ml). At arrow 3, the NaCl concentration in the eluent was raised to 0.5 M. The flow rate was 87 ml/h. Fractions indicated by the horizontal bar were collected. ——, A_{280}; ----, concn. of NaCl.

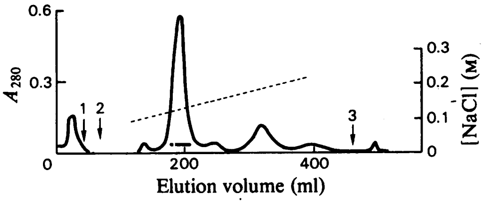

Fig. 2. *CM-cellulose (CM-52) column chromatography of Hydrophis ornatus venom from the Philippines*

Dried venom preparation (30.6 mg) was dissolved in 0.01 M-phosphate buffer, pH 6.4 (10 ml), and applied to a CM-cellulose (CM-52) column (1.5 cm × 17 cm) equilibrated wtih the same buffer. At arrow 1, the buffer was changed to 0.01 M-phosphate buffer, pH 6.4, containing 0.05 M-NaCl. At arrow 2, a linear concentration gradient elution with NaCl was applied with 200 ml of the buffer containing 0.05 M-NaCl in the mixing chamber and 200 ml of the buffer containing 0.3 M-NaCl in the reservoir. At arrow 3, the NaCl concentration in the eluent was changed to 0.5 M. The flow rate was 80 ml/h. Fractions indicated by the horizontal bar were collected. ——, A_{280}; ----, concn. of NaCl.

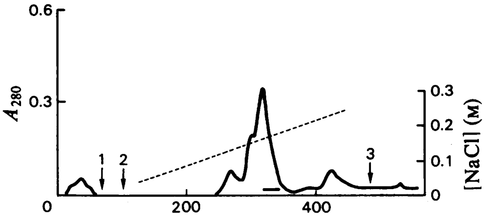

Fig. 3. *CM-cellulose (CM-52) column chromatography of Hydrophis lapemoides venom*

Dried venom (38.7 mg) was chromatographed in the same way as for the *Hydrophis ornatus* venom from the Philippines (Fig. 2). The flow rate was 80 ml/h. Fractions indicated by the horizontal bar were collected. ——, A_{280}; ----, concn. of NaCl.

Disc-gel electrophoresis of each component

Although toxins Hydrophis ornatus a and Hydrophis lapemoides a were eluted from the CM-cellulose (CM-52) column at different NaCl concentration (0.12M and 0.16M respectively), their mobilities on disc-gel electrophoresis at pH4.0 were the same. Co-electrophoresis of toxins Hydrophis ornatus a preparations from the McCluer Gulf and the Philippines venoms, toxin Hydrophis lapemoides a and toxin Astrotia stokesii a (Maeda & Tamiya, 1978) gave a single band.

Amino acid composition

The results of amino acid analysis of 24h hydrolysates of the toxins are given in Table 1. Each of alanine, valine, methionine, leucine and tyrosine was assumed to be present as one residue/molecule. Each toxin is composed of 60 amino acid residues. This number was confirmed also by the partial sequence study shown below. The compositions of toxin Hydrophis ornatus a from the McCluer Gulf and the Philippines venom preparations are the same as each other and as that of toxin Astrotia stokesii a (Table 1; Maeda & Tamiya, 1978). Toxin Hydrophis lapemoides a is different from toxin Hydrophis ornatus a in its amino acid composition and contains one phenylalanine residue/molecule, which is absent from toxin Hydrophis ornatus a.

'Fingerprint mapping' of tryptic peptides of the toxins

Because the venoms were available in small quantities, a partial sequence study was performed on toxin Hydrophis ornatus a from the McCluer Gulf venom preparation (12.6mg) and toxin Hydophis lapemoides a (10.6mg). Reduced and *S*-carboxymethylated toxin Hydrophis ornatus a (2.7mg) was digested with trypsin in 0.05M-ammonium acetate buffer, pH8.2 (0.5ml), at an enzyme/substrate ratio of 1:40 (w/w). After incubation for 16h at 37°C, the digestion mixture was freeze-dried. Freeze-drying was repeated twice after the addition of water (1.0ml each time). Reduced and *S*-carboxymethylated toxin Hydrophis lapemoides a (approx. 7mg) was digested with trypsin at an enzyme/substrate ratio of 1:50, for 16h at 37°C, in 0.05M-ammonium acetate buffer, pH7.8 (1.0ml). The digestion was stopped by the addition of 0.2M-acetic acid (2.5ml), and the digest was

Table 1. *Amino acid compositions of toxins Hydrophis ornatus a and Hydrophis lapemoides a*

Results are given in mol of amino acid residue/mol of toxin. Values in parentheses are the nearest integers. No corrections are made.

	Composition (mol of residue/mol)			
Toxin ... Amino acid	Hydrophis ornatus a*	Hydrophis ornatus a†	Hydrophis lapemoides a	Astrotia stokesii a‡
Lys	4.95 (5)	4.99 (5)	5.00 (5)	5
His	1.77 (2)	2.00 (2)	1.00 (1)	2
Arg	2.21 (2)	2.26 (2)	4.30 (4)	2
Asp	7.03 (7)	6.79 (7)	5.76 (6)	7
Thr	6.80 (7)	6.32 (7)	6.31 (7)	7
Ser	4.64 (5)	4.24 (5)	3.42 (4)	5
Glu	6.97 (7)	6.94 (7)	7.93 (8)	7
Pro	1.97 (2)	1.92 (2)	2.85 (3)	2
Gly	4.87 (5)	5.57 (5)	4.59 (4)	5
Ala	1.03 (1)	1.04 (1)	1.10 (1)	1
CyS§	6.61 (9)	8.36 (9)	7.84 (9)	9
Val	1.31 (1)	1.06 (1)	1.05 (1)	1
Met	0.95 (1)	1.02 (1)	0.76 (1)	1
Ile	2.51 (3)	2.46 (3)	1.67 (2)	3
Leu	0.99 (1)	1.00 (1)	1.00 (1)	1
Tyr	0.92 (1)	0.99 (1)	0.82 (1)	1
Phe	0 (0)	0 (0)	0.82 (1)	0
Trp‖	(1)	(1)	(1)	1
Total	60	60	60	60
M_r (formula)	6600	6600	6680	

* Preparation from the Philippines (1972) venom.

† Preparation from McCluer Gulf (1975) venom.

‡ Maeda & Tamiya (1978).

§ Cystine residues tend to give smaller values.

‖ Measured spectrophotometrically.

freeze-dried. Freeze-drying was repeated after the addition of water (1.0 ml).

A portion (300–400 μg) of each tryptic peptide mixture from toxin Hydrophis ornatus a and toxin Hydrophis lapemoides a, as well as that from toxin Astrotia stokesii a, was dissolved in 1 M-acetic acid (50 μl) and applied on a sheet of filter paper (40 cm × 40 cm). Peptide 'maps' were developed as described previously (Maeda & Tamiya, 1976). The results are shown in Fig. 4. The peptide 'map' of tryptic peptides from toxin Hydrophis ornatus a is similar to that of tryptic peptides from toxin Astrotia stokesii a, suggesting the identity of the neurotoxins.

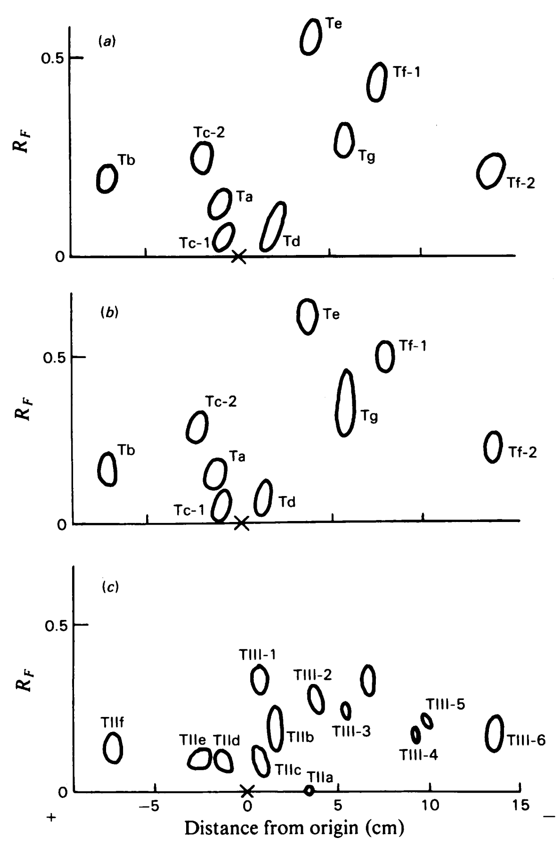

Fig. 4. *'Fingerprint maps' of tryptic digests of (a) reduced and S-carboxymethylated toxin Astrotia stokesii a, (b) reduced and S-carboxymethylated toxin Hydrophis ornatus a from the McCluer Gulf venom preparation and (c) reduced and S-carboxymethylated toxin Hydrophis lapemoides a*

All the spots are ninhydrin-positive. The colour with ninhydrin of peptides Te, Tc-2, TIII-4 and TIIb is yellow, which changes to purple gradually, indicating that the *N*-terminal residue of each of these peptides is glycine. Peptides Tg, TIII-5 and TIV are Ehrlich-positive, and peptides Tb, Tc-1, Td, Tg, TIIc, TIIe and TIIf are Pauli-positive.

Fractionation of tryptic peptides of reduced and S-carboxymethylated toxin Hydrophis ornatus a

Reduced and *S*-carboxymethylated toxin Hydrophis ornatus a (8.7 mg) was dissolved in 0.05 M-Tris/HCl buffer, pH 8.0 (0.5 ml), and digested with trypsin (0.17 mg), and the resulting peptides were fractionated on an Aminex A-4 column (0.9 cm × 55 cm) as described previously (Maeda & Tamiya, 1976). The elution pattern is given in Supplementary Publication SUP 50121. Peptides Te and Tg were pure on paper electrophoresis at pH 4.8. Peptide Ta was separated from peptide Tb by paper electrophoresis preparatively at pH 4.8. Peptide fraction Tc was evaporated to dryness, and the residue was applied to a DEAE-cellulose column (1.2 cm × 15 cm) and chromatographed with 0.01 M-phosphate buffer, pH 6.45. The NaCl concentration in the elution buffer was raised linearly to 0.2 M. Peptides Tc-1 and Tc-2 thus obtained were pure on paper electrophoresis. Fracton Tf was separated into peptides Tf-1 and Tf-2 by paper electrophoresis at pH 4.8. The amino acid compositions of these peptides (Supplementary Publication SUP 50121) can account for that of toxin Hydrophis ornatus a. The composition of each peptide is identical with that of corresponding tryptic peptide from toxin Astrotia stokesii a. The observation suggests that the amino acid sequence of toxin Hydrophis ornatus a is identical with that of toxin Astrotia stokesii a.

Fractionation of tryptic peptides of reduced and S-carboxymethylated toxin Hydrophis lapemoides a

The tryptic peptide mixture (approx. 5.5 mg) was chromatographed on a Sephadex G-25 (fine grade) column (1.3 cm × 65 cm) in 0.05 M-acetic acid and separated into four peptide fractions, TI–TIV. Fraction TII was evaporated to dryness and the residue applied to a DEAE-cellulose column (1.1 cm × 11 cm), equilibrated with 0.01 M-Tris/HCl buffer, pH 8.0. The NaCl concentration in the eluent was raised linearly to 0.5 M. The six peptides, TIIa–TIIf, that separated were pure on paper electrophoresis. Fraction TIII was separated into six peptides, TIII-1–TIII-6, preparatively by paper electrophoresis. Peptide TIV was pure on paper electrophoresis. The elution patterns of the peptides and the amino acid compositions of the isolated peptides are given in Supplementary Publication SUP 50121. The amino acid compositions of peptides TIId and TIIf are identical with the *N*-terminal and *C*-terminal segment peptides of toxin Astrotia stokesii a, namely peptides Ta and Tb, respectively. Peptides TIII-4, TIII-2 and TIIb are also identical in amino acid compositions and mobilities on paper electrophoresis at pH 4.8 and on paper chromatography in butan-1-ol–acetic acid–water (4 : 1 : 2, by vol.) with tryptic peptides TIIIe-1, TIIId and TIa from toxins Aipysurus laevis a, b and c (Maeda & Tamiya, 1976) respectively.

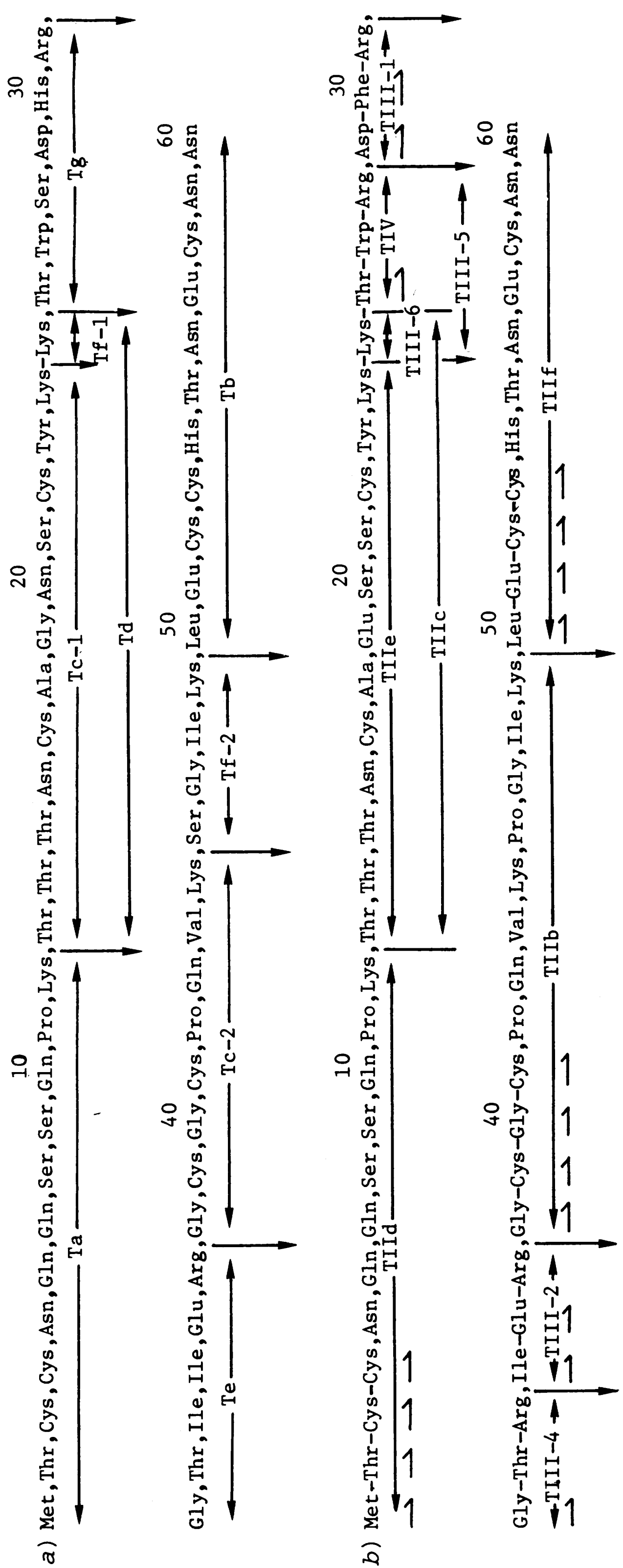

Fig. 5. *Amino acid sequences of* (*a*) *toxin Hydrophis ornatus a and* (*b*) *toxin Hydrophis lapemoides a*

Tryptic peptides are shown by the named horizontal bars. →, Amino acids detected by Edman degradation; ↓, bonds cleaved by trypsin. Sequences were deduced from the homology (see the text).

Peptide TIIc, which consists of peptides TIIe and TIII-6 linked together, and peptide TIII-5, which consists of peptides TIII-6 and TIV linked together, seem to be incomplete digestion products. The Edman degradation of peptide TIII-1 revealed its sequence to be Asp-Phe-Arg. The *N*-terminal residue of peptide TIV was a threonine. Peptide TIIe (approx. 0.05 μmol) was digested with Pronase E for 6 h in 0.05 M-Tris/HCl buffer, pH 8.0 (0.2 ml), followed with leucine aminopeptidase for 20 h. Applied directly to the amino acid analyser, the digest yielded lysine (1.11), *S*-carboxymethylcysteine (1.34), threonine (2.50), serine and/or asparagine (2.42), glutamic acid (0.70), alanine (1.00) and tyrosine (0.94); the numbers in parentheses are the molar proportions taking alanine as unity. From these results, the presence of asparagine and glutamic acid residues in this peptide was deduced. The amino acid sequence of the peptide is assumed to be identical with that of the corresponding peptide from toxins Enhydrina schistosa 4 and 5 reported by Fryklund *et al.* (1972). The amino acid sequence of toxin Hydrophis lapemoides a is thus deduced as shown in Fig. 5.

Discussion

Smith (1926), in his classical monograph on the systematics of sea snakes, included in the Family Hydrophiidae all of those marine snakes characterized by their paddle-shaped tails. More recently, Smith's classification has been revised to produce a number of Family-level classifications (Smith *et al.*, 1977; McDowell, 1967, 1969, 1972; Burger & Natsuno, 1974) in which the only common element has been the removal of the sea-kraits of the genus *Laticauda* (oviparous, semi-terrestrial) into a Family separate from that of the true sea snakes (viviparous, strictly marine aquatic).

Within the latter group, the most typical genus is *Hydrophis* (Sonnini and Latreille 1802), containing about 23 species. *Hydrophis ornatus* has long been regarded as one of the most wide-ranging members of this genus. Smith (1926) recorded it from a large number of localities between the Persian Gulf and the east coast of Australia, and recognized two subspecies: *ocellatus* from Australian waters, including the Arafura Sea, and the nominate race *ornatus* from the remainder of its range. Smith also elevated *Chitulia inornata* (Gray 1849), long regarded as a subspecies of *H. ornatus*, to full species status, recording it with certainty only from the Philippine Islands. However, Mittleman (1947) again reduced *inornatus* to a subspecies of *ornatus* and described a further race, *maresinensis*, from the East China Sea and the Ryu Kyu Archipelago.

It was against this taxonomic background that the specimens collected on the *Alpha Helix* expeditions were originally identified as *Hydrophis ornatus* from the Arafura Sea and the Philippines) and the *Hydrophis inornatus* (from the Philippines). However, the results of the amino acid sequence studies reported above contradicted these identifications, and a re-examination of these specimens has shown that only two species-level taxa are represented: *Hydrophis ornatus* (from both the Philippines and the Arafura Sea) and *Hydrophis lapemoides* (from the Philippines). The latter has long been regarded as a rather rare species that ranges from the Persian Gulf to the coasts of India and Sri Lanka, though Toriba & Sawai (1981) have more recently recorded this species from Penang, Malaysia. Our Philippines specimens therefore extend the range a further 3000 km.

The systematics of this complex will be dealt with elsewhere, at which time the status of the races *inornatus*, *ocellatus* and *maresinensis* will be reviewed. However, it is sufficient for the purpose of the present paper to point out that members of the *Hydrophis lapemoides* species-group may be readily distinguished from members of the *Hydrophis ornatus* complex by the possession of a series of cuneate scales in the infralabial series. In the sympatric Philippines populations, *H. lapemoides* is further distinguished from *H. ornatus* by its pale-centred head (uniform grey in *ornatus*; see Figures in Dunson & Minton, 1978). However, this latter difference does not appear to hold true in Malaysian populations of these two species (Toriba & Sawai, 1981). Variation in other morphological features will be described elsewhere.

On the basis of distribution, Philippine snakes in the *Hydrophis ornatus* complex should be assigned to the form *inornatus*, which, as indicated above, has variously been regarded as either a race of *ornatus* or a full species. However, on the basis of morphology, our specimens fall within the range of variation described for *H. ornatus*, to which we have assigned them, and this is strongly supported by the sequence data, which indicate that their neurotoxins are identical with those from Irian Jaya specimens of *H. ornatus*.

It is also taxonomically pertinent that toxin Hydrophis ornatus a has the same amino acid sequence as toxin Astrotia stokesii a, although the elution patterns of their total venom components are significantly different. Although the generic placement of *Astrotia stokesii* has been questioned, with McDowell (1972) assigning it to the genus *Disteira* whereas Cogger (1975) has maintained *Astrotia* as a monotypic genus, it is readily distinguished from all other sea snakes by a unique suite of morphological characters.

Conversely, six residue replacements between toxins Hydrophis ornatus a and Hydrophis lapemoides a, which are both the main neurotoxins of the venoms of their respective species, reflect a fairly

Toxin	10	20 30 40 50 60	
1. Hydrophis ornatus a	MTCCNQQSSQPKTTTNC	AGNSCYKKTWSDHRGTIIERGCGCPQVKSGIKLECCHTNECNN	Present paper
2. Astrotia stokesii a	MTCCNQQSSQPKTTTNC	AGNSCYKKTWSDHRGTIIERGCGCPQVKSGIKLECCHTNECNN	Maeda & Tamiya (1978)
3. *Hydrophis cyanocinctus* hydrophitoxin a	MTCCNQQSSQPKTTTNC	AESSCYKKTWSDHRGTRIERGCGCPQVKKGIKLECCHTNECNN	Liu & Blackwell (1974)
4. *Hydrophis cyanocinctus* hydrophitoxin b	MTCCNQQSSQPKTTTNC	AESSCYKKTWSDHRGTRIERGCGCPQVKSGIKLECCHTNECNN	Liu & Blackwell (1974)
5. *Pelamis platurus* pelamitoxin a	MTCCNQQSSQPKTTTNC	AESSCYKKTWSDHRGTRIERGCGCPQVKSGIKLECCHTNECNN	Wang *et al.* (1976)
6. Enhydrina schistosa 5	MTCCNQQSSQPKTTTNC	AESSCYKKTWSDHRGTRIERGCGCPQVKSGIKLECCHTNECNN	Fryklund *et al.* (1972)
7. Enhydrina schistosa 4	MTCCNQQSSQPKTTTNC	AESSCYKKTWSDHRGTRIERGCGCPQVKPGIKLECCHTNECNN	Fryklund *et al.* (1972)
8. *Lapemis 'hardwickii* neurotoxin	MTCCNQQSSQPKTTTNC	AESSCYKKTWSDHRGTRIERGCGCPQVKPGIKLECCHTNECNN	Fox *et al.* (1977)
9. Hydrophis lapemoides a	MTCCNQQSSQPKTTTNC	AESSCYKKTWRDFRGTRIERGCGCPQVKPGIKLECCHTNECNN	Present paper
10. Aipysurus laevis a	LTCCNQQSSQPKTTTDC	ADNSCYKKTWQDHRGTRIERGCGCPQVKPGIKLECCKTNECNN	Maeda & Tamiya (1976)
11. Aipysurus laevis c	LTCCNQQSSQPKTTTDC	ADNSCYKKTWKDHRGTRIERGCGCPQVKPGIKLECCKTNECNN	Maeda & Tamiya (1976)
12. Aipysurus laevis b	LTCCNQQSSQPKTTTDC	ADNSCYKMTWRDHRGTRIERGCGCPQVKPGIKLECCKTNECNN	Maeda & Tamiya (1976)
13. Acanthophis antarcticus c	MQCCNQQSSQPKTTTTCPGGVSSCYKKTWRDHRGTIIERGCGCPRVKPGIRLICCKTDECNN		Kim & Tamiya (1981)
14. *Laticauda semifasciata* erabutoxin b	RICFNQHSSQPQTTKTCPSGESSCYHKQWSDFRGTIIERGCGCPTVKPGIKLSCCESEVCNN		Sato & Tamiya (1971) Maeda & Tamiya (1977)
15. *Naja nigricollis* toxin α	LECHNQQSSQPPTTKTCP	GETNCYKKVWRDHRGTIIERGCGCPTVKPGIKLNCCTTDKCNN	Eaker & Porath (1967)
16. *Dendroaspis polylepis* toxin α	RICYNHQSTTRATTKSC	EENSCYKKYWRDHRGTIIERGCGCPKVKPGVGIHCCQSDKCNY	Strydom (1972)

Fig. 6. *Amino acid sequences of short-chain neutrotoxins from the venoms of snakes of the Family Elapidae*

The underlined parts of toxins 1 and 9 were sequenced in the present investigation. 1–9, Hydrophiidae; Hydrophiinae: Hydrophiini; 10–12, Hydrophiidae; Hydrophiinae: Aipysurini; 13, Hydrophiidae: Oxyuraninae: Acathophiini; 14, Elapidae: Elapini: Laticaudini; 15–16, Elapidae: Bungarinae: Najini. [Higher classification by Smith *et al.* (1977).] Key to one-letter notation for amino acids: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

Table 2. *Number of amino acid residue replacements among hydrophiid short-chain neurotoxins and some elapid short-chain neurotoxins*

1–9, Hydrophiidae: Hydrophiinae: Hydrophiini; 10–12, Hydrophiidae: Hydrophiinae: Aipysurini; 13, Hydrophiidae: Oxyuraninae: Acanthophiini; 14, Elapidae: Elapini: Laticaudini; 15–16, Elapidae: Bungarinae: Najini. [Higher classification by Smith *et al.* (1977).]

	No. of amino acid replacements															
Toxin	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Hydrophis ornatus a	0	0	4	3	3	3	4	4	6	7	7	8	14	22	19	25
2. Astrotia stokesii a	0	0	4	3	3	3	4	4	6	7	7	8	14	22	19	25
3. *Hydrophis cyanocinctus* hydrophitoxin a	4	4	0	1	1	1	1	1	3	7	7	8	14	21	19	26
4. *Hydrophis cyanocinctus* hydrophitoxin b	3	3	1	0	0	0	1	1	3	7	7	8	14	21	19	26
5. *Pelamis platurus* plamitoxin a	3	3	1	0	0	0	1	1	3	7	7	8	14	21	19	26
6. Enhydrina schistosa 5	3	3	1	0	0	0	1	1	3	7	7	8	14	21	19	26
7. Enhydrina schistosa 4	4	4	1	1	1	1	0	0	2	6	6	7	13	20	18	25
8. *Lapemis hardwickii* neurotoxin	4	4	1	1	1	1	0	0	2	6	6	7	13	20	18	25
9. Hydrophis lapemoides a	6	6	3	3	3	3	2	2	0	7	7	7	13	20	18	25
10. Aipysurus laevis a	7	7	7	7	7	7	6	6	7	0	1	2	14	23	18	25
11. Aipysurus laevis c	7	7	7	7	7	7	6	6	7	1	0	2	14	23	18	25
12. Aipysurus laevis b	8	8	8	8	8	8	7	7	7	2	2	0	14	24	18	25
13. Acanthophis antarcticus c	14	14	14	14	14	14	13	13	13	14	14	14	0	19	15	25
14. *Laticauda semifasciata* erabutoxin b	22	22	21	21	21	21	20	20	20	23	23	24	19	0	17	25
15. *Naja nigricollis* toxin α	19	19	19	19	19	19	18	18	18	18	18	18	15	17	0	22
16. *Dendroaspis polylepis* toxin α	25	25	26	26	26	26	25	25	25	25	25	25	25	25	22	0

large difference between these toxins (Fig. 6 and Table 2). The largest number of replacements observed previously among hydrophiid short-chain neurotoxins is eight. The difference suggests that the relationship between *Hydrophis ornatus* and *Hydrophis lapemoides* is much more remote than their superficial similarities would indicate.

The amino acid sequences of toxins Hydrophis ornatus a and Hydrophis lapemoides a were quoted in a previous publication (Kim & Tamiya, 1981) under the names of Hydrophis ornatus '73a and Hydrophis ornatus '75a respectively, the latter based on the erroneous assignment at that time.

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