The structure of zetekitoxin AB, a saxitoxin analog from the Panamanian golden frog _Atelopus zeteki_: A potent sodium-channel blocker

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Contributed by John W. Daly, January 23, 2004

Bufonid anurans of the genus _Atelopus_ contain both steroidal bufadienolides and various guanidinium alkaloids of the tetrodotoxin class. The former inhibit sodium-potassium ATPases, whereas the latter block voltage-dependent sodium channels. The structure of one guanidinium alkaloid, zetekitoxin AB, has remained a mystery for over 30 years. The structure of this alkaloid now has been investigated with a sample of ~0.3 mg, purified from extracts obtained decades ago from the Panamanian golden frog _Atelopus zeteki_. Detailed NMR and mass spectral analyses have provided the structure and relative stereochemistry of zetekitoxin AB and have revealed that it is an analog of saxitoxin. The proposed structure is characterized by richness of heteroatoms (C_{16}H_{25}N_{8}O_{12}S) and contains a unique 1,2-oxazolidine ring-fused lactam, a sulfate ester, and an N-hydroxycarbamate moiety. Zetekitoxin AB proved to be an extremely potent blocker of voltage-dependent sodium channels expressed in _Xenopus_ oocytes. The IC_{50} values were 280 pM for human heart channels, 6.1 pM for rat brain Ila channels, and 65 pM for rat skeletal muscle channels, thus being roughly 580-, 6.1-, and 63-fold more potent at these channels than saxitoxin.

Rightly colored bufonid anurans of the genus _Atelopus_ found in Central and South America were known to have poisonous skin secretions since the late 1800s (see ref. 1 and references therein). In 1969, Mosher and colleagues reported the presence of a potent, water-soluble, guanidinium toxin in extracts of skin of _Atelopus zeteki_, which they designated atelopidtoxin (1, 2). Later in 1975, they identified another guanidinium toxin, tetrodotoxin, as the major alkaloid in aqueous skin extracts of Costa Rican _Atelopus varius_, whereas a similar guanidinium toxin, chiquitoxin, was found as the major alkaloid in Costa Rican _Atelopus chiquiarus_ (3). The structure of chiquitoxin finally was determined in 1990 (4). The third guanidinium toxin, atelopidtoxin, was the major alkaloid in the Panamanian golden frog _A. zeteki_ (3). It was renamed zetekitoxin to indicate a unique occurrence in _A. zeteki_. Subsequent studies did not reveal the structure of the major toxic component zetekitoxin AB (LD_{50} i.p. mouse, 11 μg/kg) nor of the minor component zetekitoxin C (LD_{50} i.p. mouse, 80 μg/kg; ref. 5). Indications existed that zetekitoxin AB was a mixture of two similar compounds and, hence, the designation AB. In these early studies, zetekitoxins were chemically and pharmacologically distinguished from tetrodotoxin and saxitoxin (1–3, 5). Studies on zetekitoxins were interrupted, in part, because of designation of the frog as an endangered species, and the structures remained a mystery for >30 years. Tetrodotoxin, but not chiquitoxin nor zetekitoxin, was subsequently found in other _Atelopus_ species and in other amphitans (refs. 6–10 and references therein). Toxic steroidal bufadienolides also are present in skin of anurans of the genus _Atelopus_, including _A. zeteki_ (11). The structure of zetekitoxin AB has now been determined. Purification of ~0.3 mg from frogs, collected before restrictions, and spectral analysis showed that zetekitoxin AB (Fig. 1, I) is a saxitoxin analog with an unprecedented 1,2-oxazolidine ring-fused lactam moiety. The inhibitory effects of zetekitoxin AB on voltage-dependent sodium channels expressed in _Xenopus_ oocytes indicate that zetekitoxin AB is an extremely potent sodium channel blocker, manifold more potent than saxitoxin, but with a similar profile of activity at sodium channel isoforms.

**Methods**

**Materials.** The skins of frogs (50 skins, 10 g wet weight), presumably _A. zeteki_, collected near El Cope, Cocle, Panamá in October 1971, were kept in MeOH at −20°C until 1986. Recent mDNA data suggest that this population of frogs actually may represent a population of _A. varius_, whereas the population from El Valle studied by Fuhrman et al. (1) and other populations to the east of El Valle represent true _A. zeteki_ (A. Wisnieski, personal communication). Saxitoxin was purified from the molluscs _Spondylus butleri_ (12). For electrophysiological studies, saxitoxin was obtained from Calbiochem, Sigma, or the Marine Analytical Chemistry Standards Program of the Institute of Marine Biosciences, National Research Council of Canada (Halifax, Canada). STX from the various sources showed equivalent activities.

**Purification of Zetekitoxin AB.** Zetekitoxin AB was purified from the skin of _A. zeteki_ guided by mouse bioassay (cf., ref. 5). In 1986, the skin of frogs was homogenized and extracted with H_{2}O and then with acetic acid/H_{2}O (2:98), and the homogenate was dialyzed (30,000 M_{r} cutoff) against H_{2}O. The dialysate was concentrated by evaporation and ultrafiltered with a 10,000 M_{r} cutoff membrane. The filtrate was concentrated by lyophilization and dissolved in 1 ml of H_{2}O to be applied to a gel filtration column, Bio-Gel P-2 (1.2 × 50 cm; Bio-Rad), equilibrated with pyridine/acetic acid/H_{2}O (24:2:974, pH 6.2). The column was eluted with this solution at 1 ml/min, and the eluted toxic fraction was applied to another gel filtration column, TSK-gel G2000PW (0.75 × 30 cm; Tosoh, Tokyo) with 0.05 M acetic acid at 0.5 ml/min. The most toxic fraction (eluete at 10.2–11.1 ml) represented zetekitoxin AB. After confirmation of the purity in 1986 by TLC and 1H NMR analysis, the fraction containing zetekitoxin AB (~0.3 mg) was lyophilized and kept at −80°C until further analysis in 1999. Quantification was by 1H NMR.

Abbreviations: CID, collision-induced dissociation; ESI, electrospray-ionization; TOF, time-of-flight; HMBC, heteronuclear multiple-bond correlations; NOE, nuclear Overhauser effect.

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formed by molecular mechanics calculation (13) with SYBYL force field (14).

**NMR Data of Saxitoxin (2).** $^{13}$C NMR, $^{13}$C-$^1$H, and $^{15}$N-$^1$H gradient HMBC spectra of 2 (43 μmol in 0.4 ml of CD$_3$COOD/D$_2$O, 4:96) at 20°C were measured under the same conditions as those for zetekitoxin AB. 2: $^{13}$C NMR (150 MHz, $^{13}$CD$_3$COOD at 22.4°C): δ 33.3 (C11, after deuterium exchanged from the HMBC), 43.4 (C10), 53.8 (C6), 57.7 (C5), 63.9 (C13), 83.2 (C4), 99.2 (C12), 156.8 (C2), 158.7 (C8), 159.7 (C14); $^{15}$N NMR (60.8 MHz, partial with $^{13}$N of benzamide at 106 ppm as external reference). From the HMBC of N1/H5, N1/H13a, N1/H13b, N3/H5, N3/H10a, N3/H10b, and N7/H6 the following δ values were determined: δ 81 (N1), 103 (N3), and 88 (N7).

Preparation of the Model Compounds for ESI MS/MS (6 and 8). N-Hydroxy carbamic acid 1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl ester (6) was prepared by treatment of N-hydroxymethyl phthalimide with N,N’-carbonyldimazide in dry acetonitrile and then with hydroxylamine hydrochloride in dry pyridine (15). High-resolution ESI/TOF-MS (MeOH): [M + Na]$^+$ m/z 259.0360, calced for [C$_5$H$_4$N$_2$O$_3$Na]$^+$ m/z 259.0331. Fourier transform IR (thin film) $\nu_{max}$ 3159, 2962, 2848, 1780, 1728, 1373, 1201, 1093, 699 cm$^{-1}$; $^1$H NMR (600 MHz, CD$_3$OD, 20°C, CH$_3$D$_2$O at δ 3.30); δ 5.70 (2H, s, CH$_2$); 7.86 (2H, dd, δ = 5.3, 3.1, aromatic); 7.92 (2H, dd, δ = 5.3, 3.1, aromatic). $^{13}$C NMR (150 MHz, CD$_3$OD, $^{13}$CD$_3$OD at 49.8 ppm, 35°C): δ 62.9 (CH$_2$), 125.8, 137.0 (aromatic CH), 133.9 (aromatic quaternary), 159.8 (carbamate C = O), 169.3 (imide C = O). A formaldehyde adduct (8) was prepared from 2-imino-1-imidazolidineacetic acid with formaldehyde by stirring for 48 h at room temperature. A part of resulting mixture was diluted with MeOH or D$_2$O and used in spectral analysis. High-resolution ESI/TOF-MS: [M + H]$^+$ m/z 174.0855, calcd for [C$_5$H$_4$N$_2$O$_3$]$^+$ m/z 174.0879. $^1$H NMR (600 MHz, D$_2$O, 20°C, HDO at 4.85) δ 3.75 (2H, dd, δ = 10.2, 6.6, CH$_2$-N(CH$_2$COOH)-), 3.83 (2H, dd, δ = 10.2, 6.6, CH$_2$-N(CH$_2$COOH)-), 3.93 (2H, s, CH$_2$-COOH), 4.85 (2H, s, N-CH$_2$-OH). $^{13}$C NMR (150 MHz, D$_2$O, 35°C, $^{13}$CD$_3$COOD as external reference at 22.4 ppm). From the HMBC and heteronuclear single-quantum correlations the following δ values were determined: δ 45 (CH$_2$-N(CH$_2$COOH)-), 47 (CH$_2$-N(CH$_2$COOH)-), 48 (N-CH$_2$-COOH), 68 (N-CH$_2$-OH), 159 (guanidinium), 176 (COOH).

**Electrophysiology.** *Xenopus laevis* oocytes were isolated and injected with 20–50 nl of in vitro-transcribed sodium channel cRNA by using techniques as described (16, 17). Oocytes were studied 48–72 h after injection at room temperature (20–22°C) in a flowing-bath chamber. Transmembrane currents were measured by using two-electrode oocyte voltage-clamp (model CA1B, Dagan Instruments, Minneapolis), A/D converter (1320A, Axon Instruments, Union City, CA) and data acquisition software (AXOGRAPH 4.4, Axon Instruments) from oocytes expressing 1–4 μA of current. Sodium channel currents were elicited by applying a single 70-ms step depolarization protocol to 0 mV from −100 mV. Each step was separated by 20 s to allow for complete channel recovery. Toxin stock solutions were added to the bath solution, which consisted of 90 mM NaCl, 2.5 mM KCl, 1 mM CaCl$_2$, 1 mM MgCl$_2$, and 5 mM Hepes titrated to pH 7.2 with 1 N NaOH immediately before each experiment. The IC$_50$ values for toxin were calculated from the ratio of peak currents in the absence and presence of toxin, based on a single-site Langmuir adsorption isotherm at steady state or from the ratio of the toxin on-and off rates. Results were comparable by each technique. Toxin activity was constant over the course of the experiment.

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PNAS  |  March 30, 2004  |  vol. 101  |  no. 13  |  4347
Results

Characterization of Zetekitoxin AB. Zetekitoxin AB (1) showed the following physical and spectral characteristics. TLC: Silica Gel 60, pyridine/ethyl acetate/acetic acid/H2O, 15:7:3:6, Rf 0.57 [saxitoxin (2); Rf 0.36]; yellow fluorescent spots under UV light (365 nm) after heating with H2O2 to a temperature of 84°C, with 15N of benzamide at 106 ppm as external reference. From the HMBC of N1 and N7 (see Fig. 5, which is published as supporting information on the PNAS web site).

Structure Elucidation of Zetekitoxin AB (1). Zetekitoxin AB, purified to TLC homogeneity in 1986, now was shown to consist of an approximate 3:1 mixture of two closely related components by 1H NMR spectroscopy (Fig. 2). The structure of the major component was analyzed. The minor component may be the des-sulfate. The 1H NMR spectrum of zetekitoxin AB and its LD50 (10 μg/kg i.p. mouse) measured in 1999 were consistent with those measured in 1986.

The chemical structure of zetekitoxin AB (1) was elucidated based on NMR spectroscopy and mass spectral data. The molecular ions of zetekitoxin AB appeared at m/z 553 [M + H]+ in the positive ion mode, and at m/z 551 [M – H]– in the negative ion mode of ESI/TOF-MS. There was also an ion at m/z 473 [M – SO3 + H]+ in the positive ion mode. High-resolution ESI/TOF-MS analysis of the m/z 553 [M + H]+ peak showed that the exact mass was at m/z 553.1326, corresponding to a formula of C16H25N8O12S (calculated mass, m/z 553.1325).

The presence of an O- or N-sulfate group in zetekitoxin AB was supported by the prominent fragment ion at m/z 473 [M – SO3 + H]+ on a CID ESI-MS/MS spectrum measured by choosing the ion at m/z 553 as the precursor ion (Fig. 3A) and also by the IR band at 1,268 cm⁻¹. Existence of 10 exchangeable protons in zetekitoxin AB was indicated by the [M – 11H + 11D]+ ion (m/z 564) on an ESI-MS spectrum measured with CD3OD/CD2O (1:1), and by the fragment ion [M – SO3 – 11H + 11D]+ (m/z 484) on a CID MS/MS spectrum measured by choosing the ion at m/z 564 as the precursor ion.

The partial structure of C14–C18 in 1 and the presence of two isolated methylenes (10-CH2 and 19-CH2) and two singlet methines (H5 and H6) were deduced from 1H NMR (Fig. 2), double-quantum correlation spectroscopy, and total correlation spectroscopy. The 13C NMR spectrum (Fig. 2) showed four carbonyl or guanidinium carbon signals at 155.8, 156.5 (C2 and C13, interchangeable assignments), 158.7 (C8) and 159.2 ppm.
ions at Yotsu-Yamashita (C20), and one quaternary carbon signal-bearing oxygen at 89.4 ppm (C11). Two quaternary carbons, C4 (86.4 ppm) and C12 (97.4 ppm), were not directly observed in 13C NMR but were deduced from HMBC (Fig. 2). The heteronuclear single-quantum correlations led to assignments of all 1H and 13C signals except those of the seven quaternary carbons. The skeleton of saxitoxin (2), a major component of the paralytic shellfish toxins (18, 19), was indicated to be present in zetekitoxin AB in the same relative stereochemistry as in saxitoxin on comparison of the chemical shifts of the 13C NMR signals and the HMBC of zetekitoxin AB with those of saxitoxin. The 13C-1H HMBC of C2/H6, C4/H5, C4/H6, C4/H10β, C5/H6, C8/H5, C11/H10α, C11/H10β, C12/H5, C12/H10β, and C13/H6 (Fig. 2) and the 15N-1H HMBC of N1/H5 and N7/H6 were shared in the spectra of zetekitoxin AB and saxitoxin. These spectra are reported in Figs. 3–7. The signals of C5, C6, C10, C11, and C12 (156 ppm) of zetekitoxin AB were 3.2, 4.2, 1.8, 11.9, 5.6, 1.38, 0.17, and 0.84 ppm shifted downfield from those of saxitoxin (see 1H NMR of 2 in ref. 20) and the signal of C12 was 1.85 ppm shifted upfield (13C NMR shifts: all >1 ppm, 1H NMR shifts: all >0.1 ppm).

Zetekitoxin AB was indicated to possess three side chains at the C6, N7, and C11 positions of the skeleton of saxitoxin (2) as follows. Two cross peaks from C13 and C2 (155.8 and 156.5 ppm, interchangeable assignments) to H6 were shown on the HMBC when the column width for 13C NMR was reduced to about one-fourth of the conventional width to enhance the digital resolution (Fig. 2 Inset). The HMBC of C13/H6 and the chemical shift of C6 (55.6 ppm), typical for nitrogen-substituted methine carbons, indicated that C13 was directly connected to C6, and the C13 resonance was assigned to an amide carbonyl carbon. A similar high-field resonance of an N-hydroxy amide carbon (156.7 ppm in deuterated dimethyl sulfoxide-d6) has been reported for a cyclic hydroxamic acid, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (21). The partial structure of C14–C18 was connected to the oxygenated quaternary carbon C11 based on the HMBC of C10/H14β, C11/H14a, C11/H14β, C11/H15, C12/H14α, C14/H10α, and C14/H10β. The presence of the methyl carbamate moiety [19-CH3(O)CO]N at N7 was suggested by the chemical shifts of 19-CH3 (69.9 ppm, δH 3.99, 5.05) and C20 (159.2 ppm), and the HMBC of C5/H19α, C9/H5, C20/H19b, and N7/H19b. Isotope shifts in 13C NMR signals, as observed by the chemical shift difference between CD3COOD/D2O (4:6:90) solutions, led to partial identification of NH- or OH-bearing carbons (cf., refs. 22–24). The shifts for the quaternary carbons were not determined, except for C11, because of the low intensities of the signals. The isotope shifts for C10 and C14 were taken as 0 ppm to express the isotope shifts for all other signals >0 ppm. Significant shifts (>0.1 ppm) were observed for C5 (0.127), C6 (0.151), and C18 (0.130), whereas the shifts of the signals of C11 (0.018), C15 (0.006), C16 (0.011), C17 (0.008), and C19 (0.046) were <0.05 ppm. The shifts of C6 and C18 implied connectivity to 1-NH and 18-CH2OH, respectively. The chemical shifts of 18-CH2 (61.7 ppm, δH 3.64, 3.74), typical for hydroxyl methyl group, also supported this structure. The shift of C5 was interpreted as due to two γ-isotope effects (24) from 1-NH and 9-NH. The absence of hydroxy groups, both at N1 and N9, was judged from relatively smaller downfield shifts of C6 (2.6 ppm) and C4 (4.3 ppm) of zetekitoxin AB from those of 2, since a much larger downfield shift of C6 (11.2 ppm) was found for neosaxitoxin, the N1-hydroxy analog of saxitoxin as reported by Shimizu et al. (19).

Among eight nitrogens in the molecular formula of zetekitoxin AB, six are accounted for and analogous to the two guanidinium groups in the skeleton of saxitoxin (2). Thereby, the remaining two should be in the side chains. Except for the carbons adjacent to nitrogens in the guanidinium groups, only C13 (amide, 155.8 or 156.5 ppm), C15 (methine, 55.7 ppm, δH 4.08), and C20 (carbamate, 159.2 ppm) were assigned to the nitrogen-bearing carbons on basis of their chemical shifts. Thus, two of these carbons should be connected to the same nitrogen.
although no HMBC was observed to suggest this connectivity. The nitrogen adjacent to C15 was judged to be substituted by at least two carbons or by OH and SO₃H, based on a small isotope shift (0.006). For this reason, a linkage of C13–N–C20 was excluded, since the structures with this linkage, such as 3 (Fig. 1), should possess an NH at C15 or an OH at C17 for which isotope shifts were <0.01 ppm. Also, another structure possessing an N-OH(SO₃H) at C15, forming a spiro ring system at C11 with a six-member ring consisting of C11, C14–17, and oxygen, was not compatible with the observed NOE between H14α and H17 (Fig. 8). Further, molecular mechanics energy minimization done on 3 led to the dihedral angle of H5/H6, 143°.2, which is incompatible with an observed value close to 0° for 1. A linkage of C15–N–C20 also was excluded by the nature of such a highly strained structure. On the other hand, C13 and C15 would be located so as to form the lactam of 1 readily, if C13 and C14 were in a cis relationship. The remaining carbamate nitrogen, C20–N, would be located at the terminus of the molecule. The presence of one more ring was suggested by the degree of unsaturation. The chemical shifts of 17-CH₂ (δ 88.9 ppm, J H5/H6 close to 0 Hz, J H5/H6. A linkage of C15–N–C20 was required to satisfy the molecular formula. The relative stereochemistries of C15 and C16 were deduced as in 1 from NOE (H10β/H15, H14α/H17, H14α/H18b, and H15/H16) shown on NOE and rotating frame NOE spectroscopy (see Fig. 8, which is published as supporting information on the PNAS web site), and on NOEs shown on the NOE difference spectra, together with J values (J'H14α/H15, close to 0°; J'H14β/H13 = 7.8°; and J'H15/H16 = 7.8°). Thus, the structure of zetekitoxin AB is proposed as 1 (Fig. 1). The energy-minimized conformation of 1 deduced by molecular mechanics also is shown in Fig. 1. In this conformer, the dihedral angle of H5/H6 was predicted to be 85.6°, which is consonant with the observed value of 7.8 Hz. In toto, the data indicate that 1 is the only possible structure consonant with all the NMR analyses. Assignments of the ¹H NMR signals, for the two geminal protons H10α (δ 3.78) and H10β (δ 4.41), were based on the observed NOEs between H15 (δ 4.04) and H10β (δ 4.41). The large downfield shift (0.84 ppm) of H10β of 1 compared with that of 2 (see ¹NMR of 2 in ref. 20) is probably explained by the anisotropic effect from a carbonyl group at C13 of 1 and by the effect of substitution of C11-methylene of 2 by an O-sulfate group and a carbon chain.

The proposed structure 1 was further supported by ESIMS/MS experiments (Fig. 3 A and B). The CID MS/MS experiments were carried out in positive and negative ion modes, choosing the [M + H]⁺ ion at m/z 553 and [M − H]⁻ ion at m/z 551, respectively, as the precursor ions. In the positive ion mode, prominent fragment ions at m/z 473 [M − SO₃⁻ + H]⁻ and m/z 396 were present. The fragment ion at m/z 396 was interpreted as the iminium ion (4), probably produced by the cleavage of the HO(-CO(NH)(OH)) from the substituent at N7 and SO₃ from C11. Similar fragmentation occurred with the model compounds 6 and 8, possessing N-CH₂-O moieties (Fig. 3C). The MS/MS experiments, choosing the [M + Na]⁺ ion at m/z 259 for 6 and the [M + H]⁺ ion at m/z 174 for 8, provided the major fragment ions at m/z 160 and m/z 156, respectively, probably due to the iminium ions 7 and 9. In the negative ion mode, the MS/MS experiment for 1, choosing the [M − H]⁻ ion at m/z 551, produced a prominent fragment ion at m/z 492 (Fig. 3B). This fragment ion was interpreted as 5, produced by the cleavage of N(CO)OH from the substituent at N7. Such fragmentation patterns supported the structure of the side chain at N7 of 1 as CH₂OCONHOH.

**Pharmacology.** The effect of zetekitoxin AB (1) and saxitoxin (2) on voltage-sensitive sodium channels were assessed in *Xenopus* oocytes, injected with either human heart, rat brain IIa, or rat skeletal muscle sodium channel cRNA. Typical recordings of blockade of currents by 1 and 2 are shown in Fig. 4. The IC₅₀ values for 1 (n = 8–15) with human heart, rat brain, and rat skeletal muscle voltage-dependent sodium channels were 280 ± 3 pM, 6.1 ± 0.4 pM, and 65 ± 10 pM, respectively. The IC₅₀ values for 2 were 160 ± 14 nM, 0.97 ± 0.01 nM, and 4.1 ± 0.5 nM. Thus, zetekitoxin (1) showed the same order of selectivity as saxitoxin but was much more potent at all channels. The extremely high affinity of zetekitoxin for rat brain channels is remarkable.

**Discussion**

Structure elucidation of 1 was challenging because of seven quaternary carbons among a total of 16 carbons and a limited amount of the material (0.5 μmol), even though the initial data strongly indicated the presence of the skeleton of a known compound, saxitoxin (2). The connectivities of C13–N–C15 and C13–N–C17 in 1 were not directly confirmed by the spectral data. However, 1 appeared to be the only plausible structure that was consonant with all the spectral data. Zetekitoxin AB (1) is characterized by richness of hetero-atoms and by a 1,2-oxazolidine ring-fused lactam moiety, which apparently has never been reported in natural products. Another characteristic feature, an N-hydroxyl carbamate moiety, has been reported in a saxitoxin analog found in a xanthid crab (25). A saxitoxin analog, possessing a carbon chain, CH₂COOH, at C11, has been identified in another species of xanthid crab (26). In addition, saxitoxin analogs, having a sulfate ester at C11, are known to be the major components of paralytic shellfish toxins, called gonaytoxins (27). The presence of an analog of saxitoxin in an amphibian is unprecedented.

Further studies may reveal a much wider distribution in nature of saxitoxin analogs similar to the very wide distribution of tetrodotoxin and analogs (28, 29). Both marine bacteria (*Morax-
ella; ref. 30) and freshwater cyanobacteria (Cylindrospermopsis, Aphanizomenon, Anabaena, and Lyngbya species; refs. 20 and 31–33) have been found to produce saxitoxin. Recently, evidence that saxitoxin is produced by certain anaerobic bacteria (Enterobacter and Klebsiella species) has been reported (34). Such bacteria were proposed to cause bovine paraplegic syndrome. Thus, bacteria may be found to be responsible for the zetekitoxin because such frogs raised in captivity have no detectable tetrodotoxin (35).

The remarkable potency of zetekitoxin AB in blocking voltage-dependent sodium channels was to some extent unexpected. Initial studies had suggested that zetekitoxin (atelopidtoxin) differed in actions from tetrodotoxin (36). Thus, it was concluded from these initial studies that zetekitoxin differed in actions from tetrodotoxin and saxitoxin. Later, it was shown that zetekitoxin (ate-lopidtoxin) blocked the effect of veratridine, a sodium channel activator in guinea pig brain slices, as did tetrodotoxin and saxitoxin (37). Our data resolve this quandary and provide the chemical identification of zetekitoxin as a saxitoxin analog. Zetekitoxin AB shows an affinity profile similar to that of saxitoxin for brain, heart, and muscle sodium channels. Nevertheless, zetekitoxin AB has considerably higher affinity for each isoform than saxitoxin. Further studies on this potent and selective neurotoxin are warranted should synthetic or bacterial sources be developed.

This work is dedicated to the memory of the late Harry S. Mosher. We thank Dr. M. Satake (Tohoku University) for discussions and Dr. H. Naoki (Suntory Institute for Bioorganic Research, Osaka) for measurement of ESI-TOF MS/MS spectra. This work was supported by Grants-in-Aid 12045210 from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and 14560080 from the Japan Society for the Promotion of Science and funding from the Fujisawa Foundation and the Suntory Institute for Bioorganic Research (to M.Y.-Y.), a Scientist Development Award from the National American Heart Association (to S.C.D.), a Veterans Affairs MERIT award (to S.C.D.), National Institutes of Health Award HL64828 (to S.C.D.), and a Southeast Affiliate American Heart Association postdoctoral fellowship award (to A.P.).