Biosynthesis of Marine Toxins

Jonathan R. Chekan[1], Timothy R. Fallon[1], Bradley S. Moore[1,2]

[1]Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA, USA

[2]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, CA, USA

Corresponding author: Moore, Bradley S (bsmoore@ucsd.edu)

Abstract

Throughout history, humans have encountered natural toxic chemicals from the ocean environment, often through contaminated seafood. While marine toxins can be harmful to human health and devastate local environments when they are produced during algal bloom events, they are also important biochemical research reagents and drug leads in medicine. In spite of their long history, the biosynthetic origin of many well-known marine toxins has remained elusive. New biosynthetic insights have shed light on the chemical transformations that create the complex structures of several iconic oceanic toxins. To that end, this review highlights advances made in the biosynthetic understanding of five important environmental toxins of marine origin: domoic acid, kainic acid, saxitoxin, tetrodotoxin, and polyether polyketides such as brevetoxin.

Introduction

Natural toxins from the marine environment have long fascinated scientists for their extraordinary chemical structures and potent biological properties. Marine neurotoxins in particular have revealed the function and modulated the activity of numerous cellular proteins germane to life such as ion channels [1] and receptor proteins [2,3]. At the same time, toxin-producing oceanic harmful algal blooms (HABs) continue to dramatically harm the environment, our health, and livelihood [4–6], as witnessed most recently during the devastating *Karenia brevis* bloom off Southwest Florida in 2019. Unlike freshwater systems wherein cyanobacteria are generally responsible for large scale production of toxins [7], the major producers of marine neurotoxins are eukaryotic organisms, such as dinoflagellates and diatoms, that have genomes many orders of magnitude larger. This difference has slowed our general understanding of how marine toxins are produced at the molecular level due to the dearth of genomic data and tools available to the marine community. The freshwater cyanobacterial HAB community, on the other hand, has firmly established biosynthetic pathways to most major cyanotoxins [8], which was aided by the smaller genomes and recognizable gene clusters of cyanobacteria. As such, cyanobacterial toxin transcription can now be monitored in the environment because the biosynthetic genes have been identified [9].

In spite of the challenges in studying marine toxin biosynthesis, substantial progress has been made, especially in connecting toxins to their biosynthetic genes.

This review focuses on advances made in understanding the biosynthetic pathways of domoic acid, kainic acid, saxitoxin, tetrodotoxin, and large polyether compounds such as brevetoxin. Studies on the production of these toxins have revealed unusual and interesting enzymology. Furthermore, these new insights may facilitate biocatalytic production methods and improved environmental monitoring approaches in the years to come.
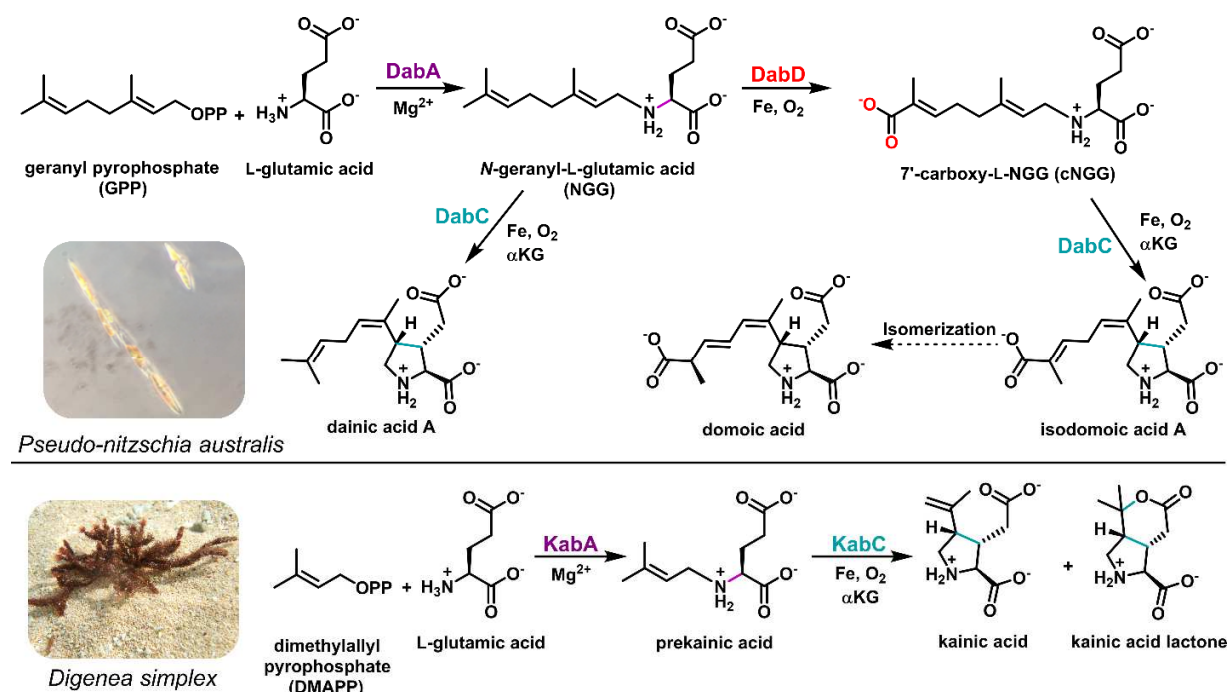
Domoic acid

Domoic acid is a potent neurotoxin produced primarily by diatoms of the *Pseudo-nitzschia* genus along with a few red algae (Figure 1). It acts as an agonist of ionotropic glutamate receptors (iGluRs), promoting an influx of calcium into neurons that ultimately leads to overstimulation and excitotoxicity [10,11]. Even though domoic acid was first discovered in the 1950s from the red algae *Chondria armata* [12], it rose to prominence during a major *Pseudo-nitzschia multiseries* diatom bloom in 1987 in Prince Edward Island, Canada [13]. During this event, filter feeding mussels bioaccumulated domoic acid and human consumption of the contaminated mussels lead to Amnesic Shellfish Poisoning, which is characterized by memory loss, seizures, and even death in extreme cases [11]. Since that time, domoic acid concentrations in shellfish and the presence of *Pseudo-nitzschia* blooms are closely monitored around the globe.

Due to the important human health implications, the route of domoic acid biosynthesis has been a focus of research for decades. While early isotopic feeding studies demonstrated that domoic acid is likely derived from geranyl diphosphate (GPP) and L-glutamic acid [14,15], the enzymes responsible for the biosynthesis were unknown. To answer this question, Brunson and McKinnie *et. al.* employed a transcriptomic based approach to identify the genes upregulated under domoic acid producing culture conditions [16"]. Surprisingly, four of the ten most upregulated genes were clustered together in the genome. The genes were bioinformatically predicted to be a terpene cyclase (*dabA*), hypothetical protein (*dabB*), α-ketoglutarate (αKG) dependent dioxygenase (*dabC*), and cytochrome P450 oxidase (*dabD*). Each of the four putative biosynthetic enzymes could be heterologously expressed and in vitro activity assays were used to assemble the pathway (Figure 1). The first committed step of the biosynthesis is catalyzed by DabA which performs *N*-geranylation of L-glutamic acid to produce *N*-geranyl-L-glutamic acid (NGG) using GPP as the prenyl donor [16"]. Recent work has confirmed the unexpected bioinformatic annotation of DabA by demonstrating that it is structurally similar to members of the terpene cyclase family [17"], making it the first known *N*-prenyltransferase in this ubiquitous family of enzymes. After formation of NGG, the cytochrome P450 enzyme DabD then catalyzes three successive oxidations of the 7' carbon of the prenyl chain to generate 7'-carboxy-L-NGG. Finally, DabC, an αKG-dependent dioxygenase, catalyzes ring closure by stereoselectively forming a new carbon-carbon bond to yield the product isodomoic acid A, a previously isolated natural

product [18]. A final isomerization step is predicted to convert isodomoic acid A to domoic acid, however the responsible enzyme has yet to be identified.

While in vitro enzymatic activity clearly demonstrates that the *dab* cluster is responsible for domoic acid production, isolation and feeding studies also support the biosynthetic proposal [19·]. Large scale isolation of compounds from the red algae *C. armata* identified several putative domoic acid metabolites, most notably NGG and dainic acid [19·], a proposed off pathway DabC enzymatic product of NGG [16··] (Figure 1). Moreover, feeding studies with [^{15}N, D]-labeled NGG showed *P. multiseries* incorporated the labels into domoic acid, again supporting NGG as an authentic intermediate [19·].

Figure 1: Biosynthesis of domoic acid and kainic acid. Photo credit: *Pseudo-nitzschia australis* from Monica Thukral (University of California, San Diego) and *Digenea simplex* from Toshiaki Teruya (University of the Ryukyus). Throughout the review, compounds are shown in their physiological charge states for consistency, even though domoic acid and kainic acid are not often depicted in this manner

Kainic acid

In addition to domoic acid, kainic acid is the other prominent member of the kainoid class of natural products found in marine environments (Figure 1). Kainic acid was originally isolated from the marine red algae *Digenea simplex.* It shares the canonical pyrrolidine core with domoic acid but has a shorter moiety at the C4 position

[20,21]. Similar to domoic acid, kainic acid acts as an iGluR receptor agonist, but is comparatively less potent [22]. Instead, kainic acid has been used clinically to treat parasitic worm infections [23,24], reflecting the centuries-long use of *D. simplex* as an anthelmintic remedy.

While feeding studies suggested a route of biosynthesis for domoic acid prior to the discovery of the biosynthetic genes, virtually no work had been completed to elucidate the kainic acid biosynthetic pathway. Instead, the structural similarities between domoic acid and kainic acid suggested a conserved route of biosynthesis. By using whole genome sequencing, genes homologous to both *dabA* and *dabC* were discovered from two kainic acid producing red algae, *D. simplex* and *Palmaria palmata*, and named *kabA* and *kabC* [25··]. Unexpectedly, both *D. simplex* and *P. palmata* clustered their kainic acid biosynthetic genes, which suggests that biosynthetic gene clustering may also be a feature in red algae. Heterologous expression of *kabA* and *kabC* in *Escherichia coli* and subsequent in vitro activity assays demonstrated that KabA catalyzes the *N*-prenylation of L-Glu using dimethylallyl diphosphate (DMAPP) as a prenyl donor to yield the intermediate prekainic acid [25··] (Figure 1). KabC, an αKG-dependent dioxygenase, then cyclizes prekainic acid to generate kainic acid. Notably, KabC also forms the natural product kainic acid lactone [25··,26] and the ratio of kainic acid to kainic acid lactone appears to vary depending on the specific KabC ortholog. Additional isolation studies demonstrated the presence of prekainic acid in *D. simplex*, further supporting the in vitro demonstrated kainic acid biosynthetic route [27].

As kainic acid is an important neurological tool and has previously been used as an anthelmintic agent [23,24,28], there has been significant interest in developing cost-effective production methods. While over 70 syntheses have been published, most remain low yielding or challenging to employ industrially [29]. Discovery of the biosynthetic gene cluster enabled an efficient two-step biotransformation of kainic acid that is both scalable and economical [25··].
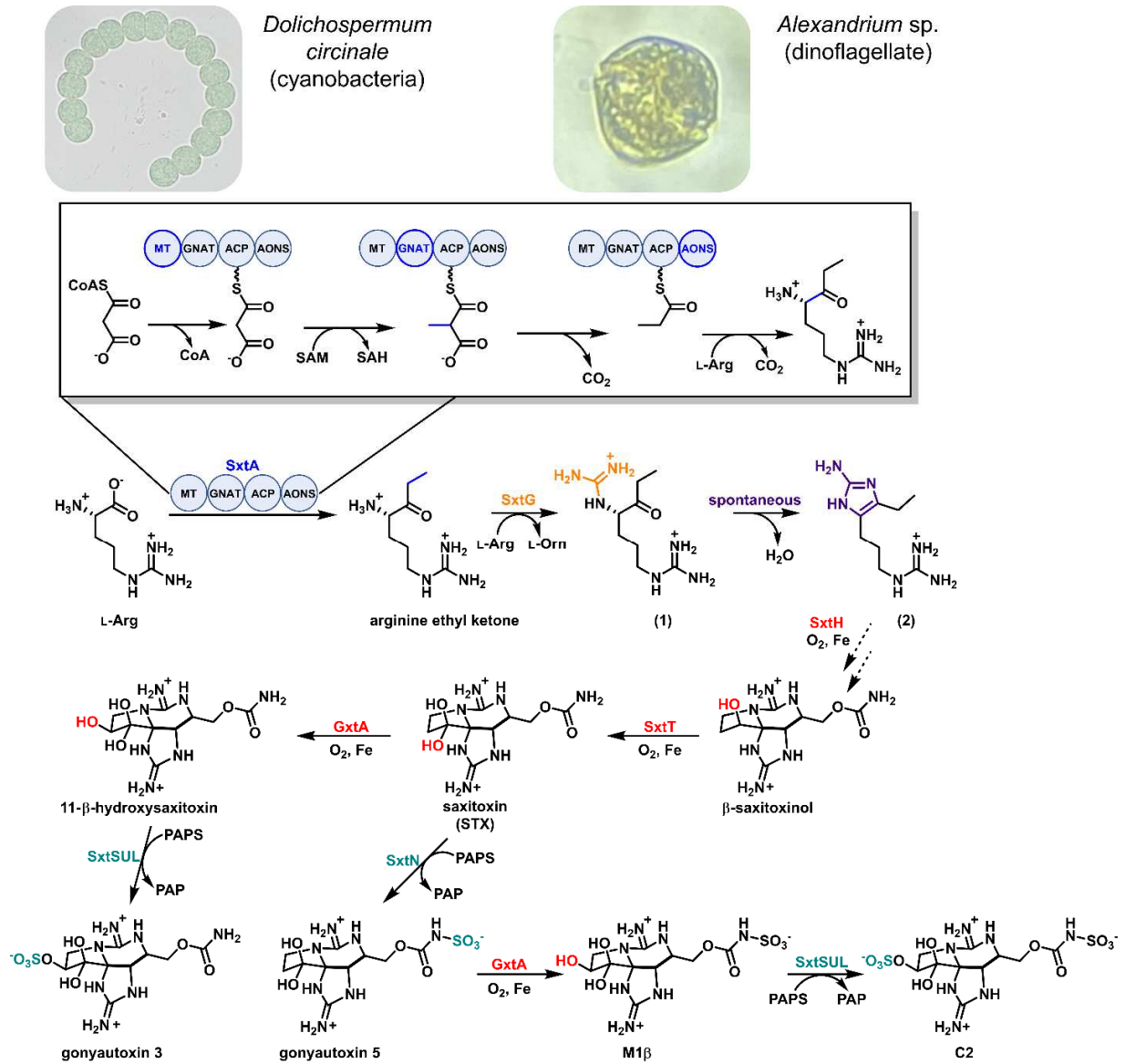
Saxitoxin

Paralytic shellfish toxins (PSTs) are a family of over 50 related alkaloid compounds that all share two guanidine moieties [30] and are produced by a diverse array of marine dinoflagellates, freshwater cyanobacteria, and brackish water cyanobacteria. PSTs exert their toxicity by binding to voltage-gated sodium channels and blocking them [31,32]. This is a major concern because when these PST producing microalgae bloom, they are consumed by filter feeders that bioaccumulate the toxins. Subsequent human ingestion of the contaminated seafood leads to paralytic shellfish poisoning, which is characterized by tingling of extremities, difficulty breathing, paralysis, and even death [32]. In addition to human poisoning events, blooms of PST producing algae also lead to death of wildlife, livestock and pets [30,33].

The founding member of the PST family, saxitoxin (STX), was originally isolated in 1957 [34], and the putative gene cluster was discovered in 1998 by the Neilan laboratory [35] (Figure 2). Similar genes were also found in marine dinoflagellates [36, 37], suggesting an interdomain horizontal gene transfer event that lead to a conserved route of biosynthesis across domains of life [38]. While metabolite characterization [39, 40], cell lysate activity assays [41], and feeding studies of labeled precursors and intermediates [40, 42, 43] had been completed, no definitive work linked the proposed cluster to enzyme activity. Recently though, substantial insights into the biosynthetic pathway and mechanisms of catalysis have been detailed. The Narayan laboratory demonstrated that biosynthesis of saxitoxin is initiated by SxtA, a polyketide synthase (PKS)-like enzyme with four domains [44··] (Figure 2). The ACP domain of SxtA is first loaded with malonyl-CoA by either the GNAT domain or a trans-acting acyltransferase protein. After loading, the methyltransferase domain catalyzes *C*-methylation to form methylmalonyl-ACP, which is subsequently decarboxylated by the GNAT domain to generate propionyl-ACP. Finally, a pyridoxal 5'-phosphate (PLP)-dependent 8-amino-7-oxononanoate synthase (AONS) domain catalyzes coincident decarboxylation of arginine and addition of the propionyl moiety to generate arginine ethyl ketone, the first committed intermediate in the biosynthesis of PSTs. Notably, this intermediate has been detected from both cyanobacterial and dinoflagellate producers of PSTs [39].

The next enzyme in the pathway, SxtG, is an amidinotransferase that catalyzes the transfer of the amidino group from arginine to the α-amine of arginine ethyl ketone [45·] (Figure 2). The unnamed product, **(1)**, then undergoes a spontaneous cyclodehydration reaction to produce **(2)**. A series of uncharacterized reactions that involve intramolecular cyclization, carbomylation, and hydroxylation are suggested to advance **(2)** to the natural product β-saxitoxinol. While the details of these transformations are still under investigation, the Rieske oxygenase SxtH is proposed to catalyze the β-hydroxylation of a linear arginine derivative intermediate [46]. After formation of β-saxitoxinol, a second Rieske oxygenase, SxtT, hydroxylates at the C12 position to generate the final saxitoxin structure [46]. A series of hydroxylations and sulfurylations further elaborate the saxitoxin molecule to produce the neosaxitoxin, gonyautoxin, C-toxin, and M-toxin series of natural products. To generate this suite of PSTs, it has been demonstrated that the Rieske oxygenase GxtA catalyzes hydroxylation at the C11 position [47], while the sulfotransferases SxtN and SxtSUL target the amide nitrogen and the C11 hydroxyl group, respectively [47,48·]. As three different Rieske oxygenases are found in the biosynthesis of PSTs, the basis for both substrate and regio-selectivity remains an outstanding question. Recent structural studies have begun to identify the biochemical determinants that lead to selectivity in SxtT and GxtA [49].

Although PSTs are found in both freshwater cyanobacteria and marine dinoflagellates, all the described in vitro biochemical work was completed with

cyanobacterial enzymes. While homologous genes are found in dinoflagellates [36, 37] and extensive isolation and characterization of intermediates has been accomplished [39, 43, 50], looking ahead, work is needed to demonstrate whether the details of the biosynthetic pathway are shared between dinoflagellates and cyanobacteria.

Figure 2: Biosynthesis of saxitoxin and other PSTs. Abbreviations are as follows: MT (methyltransferase), GNAT (GCN5-related *N*-acetyltransferase), ACP (acyl carrier protein), AONS (8-amino-7-oxononanoate synthase), PAPS (3′-phosphoadenosine-5′-phosphosulfate), and PAP (3′-phosphoadenosine-5′-phosphate). Photo credit: *Dolichospermum circinale* (formerly *Anabaena circinalis*) from Takashi Minowa (Tohoku University) and *Alexandria* sp. from April Lukowski (University of Michigan).
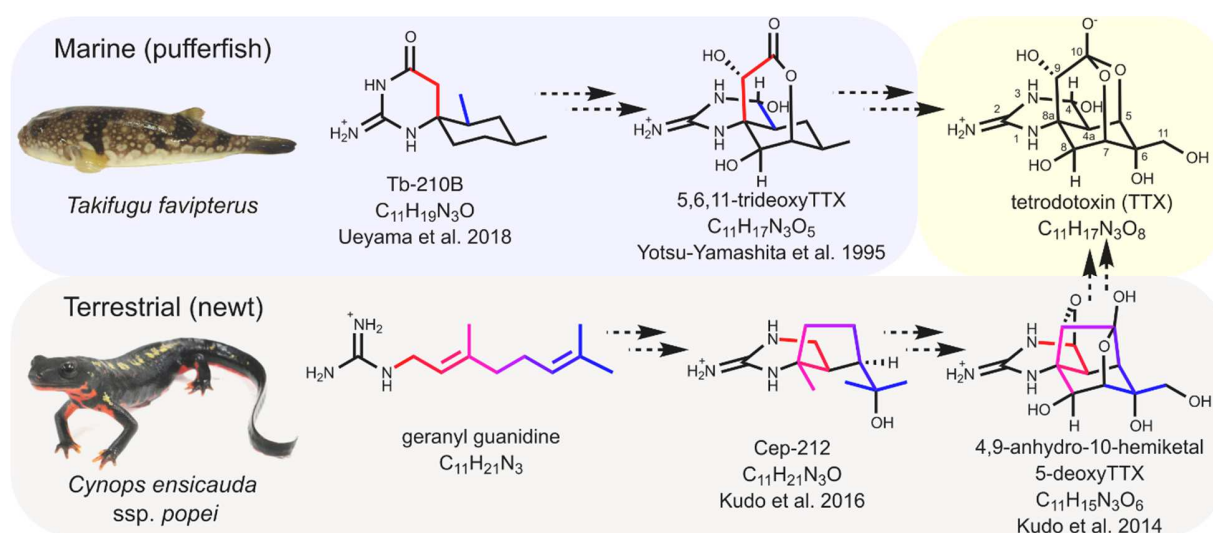
Tetrodotoxin

Tetrodotoxin (TTX) is a potent paralytic sodium channel blocker with fascinating dioxaadamantane and cyclic guanidine structural features (Figure 3) [51]. Poisoning usually happens after ingestion of tetrodotoxin-laden pufferfish, resulting in muscle paralysis and, in severe cases, death. Tetrodotoxin is found as a concentrated defensive compound in various lineages of marine and terrestrial animals, with pufferfish and newts being the most well studied examples, respectively [52].

Although tetrodotoxin has been the subject of focused biosynthetic inquiry for decades, its assembly remains unclear, and no biosynthetic gene has yet been identified. This unsolved biosynthetic mystery has been exacerbated by a lack of a reproducible organism and condition wherein tetrodotoxin can be robustly produced in the laboratory. The diverse distribution amongst animals, combined with its unusual structure, has suggested a bacterial origin coupled with either dietary acquisition of tetrodotoxin by animals from exogenous sources [53–55], or endogenous acquisition via bacterial symbioses [56,57]. Several dozen bacteria from distantly related phyla, including most recently a *Bacillus* (Firmicute) strain from *Cephalothrix* ribbon worms [58˙,59] and *Pseudomonas* (Proteobacteria) strains from *Taricha* newts [60˙], have been isolated from tetrodotoxin-containing animals and reported to produce low levels of the toxin, often at sub ng/mL concentrations [61]. Unfortunately, isolated bacterial strains have so far proven unreliable. No definitive stable isotope feeding study, production curve, or genomic signature of tetrodotoxin biosynthesis has yet been established [62], leaving still open questions about its origin and biosynthesis.

Given tetrodotoxin's unprecedented chemical structure, numerous hypotheses concerning its assembly have invoked a wide array of pathways involving carbohydrate, shikimate, terpene, and polyketide precursors. An intriguing biosynthetic proposal by Yotsu-Yamashita and coworkers recently introduced a monoterpene origin based on the co-occurrence of a series of guanidino compounds from toxic newts [63–65˙˙]. The most telling proposed tetrodotoxin biosynthetic intermediates include guanidino-containing monoterpenes and hemiketal-type tetrodotoxins that suggest extensive late-stage terpene oxidative transformations (Figure 3). Although these proposed tetrodotoxin intermediates, including 4,9-anhydro-10-hemiketal-5-deoxyTTX, can be accumulated through diet by tetrodotoxin-free *Cynops* newts raised in captivity, they are not further metabolized to or from tetrodotoxin [66˙]. Interestingly, the newt-based guanidino compounds are not shared by pufferfish, which instead accumulate bicyclic guanidino compounds of unknown origin [67˙˙] and imply an orthogonal pathway in marine organisms (Figure 3).

From a bacterial biosynthesis perspective, once the strain reliability of tetrodotoxin production is solved, then preliminary identification of tetrodotoxin biosynthetic genes via genomics or genetics should not be far behind. The terrestrial and marine animal-accumulation studies suggest orthogonal biosynthetic pathways

7

assuming the co-occurring guanidino compounds do actually represent authentic biosynthetic intermediates. As such, this intriguing small molecule still remains a mystery after several decades since its characterization in 1964 [51]. Once solved, many additional chemical ecology riddles relating to toxin acquisition and function may next be in line to unmask.

Figure 3: Hypothetical routes for tetrodotoxin (TTX) (top-right) biosynthesis, as supported by animal-accumulated candidate-biosynthetic intermediates in marine environments (top) [67**-69], exemplified by the pufferfish *Takifugu favipterus,* photo credit: Mari Yotsu-Yamashita (Tohoku University), and terrestrial environments (bottom) [63-65**], exemplified by the newt *Cynops ensicauda* ssp. *popei*, photo credit: Yuta Kuda (Tohoku University). The red-to-blue color gradients indicate the hypothetical atom-to-atom correspondence between putative pathway intermediates.

Brevetoxin and other polyether toxins

Polyether toxins from marine microalgae are distinguished from all other toxins reported in this article by their massive chemical structures. A suite of potent neurotoxins such as brevetoxins (>850 Da), ciguatoxins (>1000 Da), palytoxins (>2680 Da), and maitotoxins (>3400 Da), join medicines like the tubulin-binding mitotic inhibitor halichondrin B (>1100 Da; source of the FDA-approved derivative eribulin), as some of the iconic marine polyether compounds that have inspired biosynthetic chemists for decades [70]. Dinoflagellate microalgae, such as *Karenia* (order Gymnodiniales), *Gambierdiscus*, and *Ostreopsis* (both order Gonyaulacales), are the primary producers of many of these large polyether toxins [71–75], but there are also reports of polyether biosynthesis by marine haptophyte microalgae such as in the case of the prymnesin family of fish toxins [76,77].

Inspection of the large hydrocarbon structure of the ladder-frame polyether toxins alongside a suite of isotopic tracer experiments has strongly supported a polyketide

origin. Moreover, these studies support that polyethers are authentic products of the dinoflagellates rather than from associated symbionts [78-80]. The stereochemical regularity of the *trans*-fused ring-systems of these compounds, as is the case of the 11-fused cyclic-ether scaffold of brevetoxin B, led to the early hypothesis that polyepoxide precursors are converted via electrophilic epoxide-opening cascade reactions [82] (Figure 4a). Biomimetic synthetic chemistry experiments have corroborated the plausibility of such a cascade [83], but notably this scheme, while inarguably elegant, remains unproven [83].

While there are some similarities with bacterial or fungal polyketide biosynthesis, substantial differences exist in the dinoflagellate polyether synthesis system. Unlike bacterial polyketide biosynthesis, the utilized starter and extender molecules are rather limited, consisting of only glycine, glycolate, and acetate (or malonate). Formation of the carbon backbone is also mechanistically unusual, with Favorskii-like rearrangement mediated deletions of internal carbons, β-alkylation, pseudo-α-alkylation, the 'odd-even' rule for methylation, and the presence of initiator rings for epoxidation cascades. Readers are directed to the following papers for excellent summaries of this unusual chemistry, and of the stable isotope tracing results of dinoflagellate polyethers [80,84].

Although there has been substantial progress in understanding dinoflagellate polyether biosynthesis from the perspective of precursor incorporation [80], the genetic basis of dinoflagellate polyether biosynthesis has thus far been experimentally intractable. Dinoflagellates have very large genomes, a high prevalence of modified nucleotides [85], and genes in repetitive tandem gene arrays that undergo common *trans*-splicing [86]. Alongside their complex genome architecture, dinoflagellates also mostly lack transcriptional regulation and instead seem to rely largely on translational or post-translational regulation [74]. Therefore, recent studies have instead focused on *de novo* transcriptome assembly for identification and cataloging of acetyl-CoA carboxylase [87] and PKS genes that presumably contribute to the synthesis of these compounds. Although several studies [71-73] have reported both single domain (Type II) or multi domain (Type I) PKS or fatty acid synthase-like gene sequences from toxin-producing dinoflagellates species, the unexpectedly large number of identified genes and lack of clear correlations with toxin content has so-far prevented a subset of these genes from being definitively linked to toxin biosynthesis. Van Dolah and colleagues recently reported a promising candidate in a 7-module (10,000+ amino acid) PKS encoding transcript from the ciguatoxin producing *Gambierdiscus polynesiensis* [88ꞏ](Figure 4b). While this particular trans-acyltransferase PKS would not be large enough to synthesize the entire ciguatoxin backbone, this is the latest sequencing result to illuminate the potential of multimodular assembly line biosynthesis in a polyether toxin producing dinoflagellate.

9
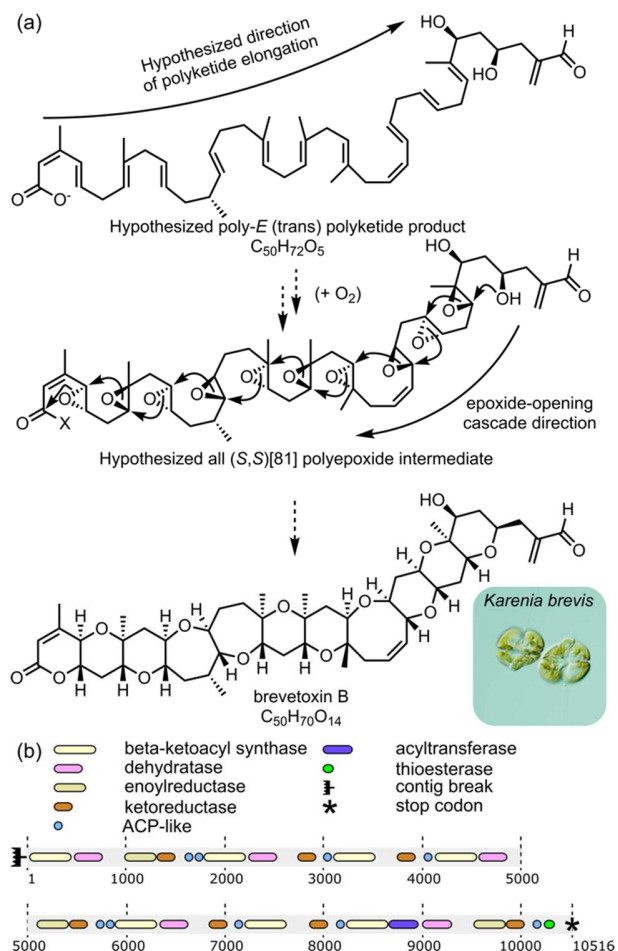
Figure 4: (a) Epoxide-opening cyclization cascade for brevetoxin B, akin to that first proposed by Nakanishi [82]. Unlike Nakanishi, we show (*S,S*) instead of (*R,R*) epoxides, and also show the epoxidation cascade proceeding in the opposite direction (away from heptadienal group). This modified scheme is consistent with more recent hypotheses that the polyepoxide stereochemistry could be (*S,S*) [80], and that the direction of the epoxidation cascade could be opposite the direction of polyketide extension [81]. Inset: The dinoflagellate *Karenia brevis*, a producer of brevetoxins. Photo Credit: Florida Fish and Wildlife Conservation Commission (CC BY-NC-ND 2.0). **(b)** Domain structure of the 10,000+ amino acid modular PKS (NCBI accession: MT165590.1) reported by Van Dolah and colleagues [88•].

Conclusions and Outlook

The examples discussed in this review highlight the substantial strides recently accomplished toward elucidating the biosynthetic pathways for several prominent marine toxins. They also highlight some of the continuing challenges that have mystified the marine toxin field for decades. In the case of the neurotoxic kainoids (domoic acid and kainic acid) and saxitoxins, biosynthetic progress over the last few years has been

significant. Major portions of their pathways have now been rigorously established and have identified new biosynthetic reactions. Moreover, the surprising discovery that the domoic acid biosynthesis genes were clustered and transcriptionally linked in toxic *Pseudo-nitzschia* diatoms opens the possibility that other eukaryotic microalgal toxin biosynthesis genes may be similarly organized.

While less is known about how tetrodotoxin is assembled, noteworthy advances were taken in recent years with reports on the isolation of tetrodotoxin-producing bacteria from both marine and terrestrial animal sources. If true, genome-based experiments may soon allow for the interrogation of the compelling monoterpenoid biosynthetic pathways proposed for the construction of tetrodotoxin. The massive polyether toxins of microalgae like brevetoxin and ciguatoxin, however, are still an enigma. Transcriptomic experiments continue to provide encouraging data on a plethora of PKS genes in dinoflagellates, many even encoding multimodular assembly line proteins akin to those well-known from classical bacterial polyketide biosynthesis. The very large genome sizes and complex genetics of dinoflagellates has precluded the "last-mile" problem of definitively identifying the responsible biosynthetic genes. Experiments which focus more on the biochemistry of biosynthesis, such as cell-free lysate biochemistry, activity-guided fractionation, and structural elucidation of trapped chain-elongation intermediates, may be orthogonal approaches that could produce alternative insights into the solution of this difficult problem.

With rapid advances in scientific knowledge and methodology, we anticipate that the coming years will continue to see exciting progress in bringing to life the biosynthetic stories of some of nature's most fascinating chemicals, the marine toxins.

Acknowledgements

References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:
• of special interest
•• of outstanding interest

1.   Bajaj S, Ong ST, Chandy KG: **Contributions of natural products to ion channel pharmacology**. *Nat Prod Rep* 2020, **37**:703-716.

2. Herndon RM, Coyle JT: **Selective destruction of neurons by a transmitter agonist**. *Science* 1977, **198**:71–72.

3. Lodge D: **The history of the pharmacology and cloning of ionotropic glutamate receptors and the development of idiosyncratic nomenclature**. *Neuropharmacology* 2009, **56**:6–21.

4. Mello FD, Braidy N, Marçal H, Guillemin G, Nabavi SM, Neilan BA: **Mechanisms and effects posed by neurotoxic products of cyanobacteria/microbial eukaryotes/dinoflagellates in algae blooms: A review**. *Neurotox Res* 2018, **33**:153–167.

5. Grattan LM, Holobaugh S, Morris JG: **Harmful algal blooms and public health**. *Harmful Algae* 2016, **57**:2–8.

6. Anderson DM, Cembella AD, Hallegraeff GM: **Progress in understanding harmful algal blooms: Paradigm shifts and new technologies for research, monitoring, and management**. *Ann Rev Mar Sci* 2012, **4**:143–176.

7. Buratti FM, Manganelli M, Vichi S, Stefanelli M, Scardala S, Testai E, Funari E: **Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation**. *Arch Toxicol* 2017, **91**:1049–1130.

8. Cullen A, Pearson LA, Mazmouz R, Liu T, Soeriyadi AH, Ongley SE, Neilan BA: **Heterologous expression and biochemical characterisation of cyanotoxin biosynthesis pathways**. *Nat Prod Rep* 2019, **36**:1117–1136.

9. Al-Tebrineh J, Pearson LA, Yasar SA, Neilan BA: **A multiplex qPCR targeting hepato- and neurotoxigenic cyanobacteria of global significance**. *Harmful Algae* 2012, **15**:19–25.

10. Stewart GR, Zorumski CF, Price MT, Olney JW: **Domoic acid: A dementia-inducing excitotoxic food poison with kainic acid receptor specificity**. *Exp Neurol* 1990, **110**:127–138.

11. Pulido OM: **Domoic acid toxicologic pathology: A review**. *Mar Drugs* 2008, **6**:180–219.

12. Takemoto T, Daigo K: **Constituents of *Chondria armata***. *Chem Pharm Bull* 1958, **6**:578b–580.

13. Wright JLC, Boyd RK, De Freitas ASW, Falk M, Foxall RA, Jamieson WD, Laycock MV, McCulloch AW, McInnes AG, Odense P, et al.: **Identification of domoic acid, a neuroexcitatory amino acid, in toxic mussels from eastern Prince Edward Island**. *Can J Chem* 1989, **67**:481–490.

14. Douglas DJ, Ramsey UP, Walter JA, Wright JLC: **Biosynthesis of the neurotoxin**

domoic acid by the marine diatom *Nitzschia pungens forma multiseries*, determined with [^{13}C]-labelled precursors and nuclear magnetic resonance. *J Chem Soc, Chem Commun* 1992, **9**:714-716.

15. Ramsey UP, Douglas DJ, Walter JA., Wright JL: **Biosynthesis of domoic acid by the diatom *Pseudo-nitzschia multiseries***. *Nat Toxins* 1998, **6**:137–146.

••16. Brunson JK, McKinnie SMK, Chekan JR, McCrow JP, Miles ZD, Bertrand EM, Bielinski VA, Luhavaya H, Oborník M, Smith GJ, et al.: **Biosynthesis of the neurotoxin domoic acid in a bloom-forming diatom**. *Science* 2018, **361**:1356–1358.

A transcriptomics-based approach enabled discovery of the domoic acid biosynthetic cluster and initial characterization of the biosynthetic enzymes from *Pseudo-nitzschia* diatoms.

•17. Chekan JR, McKinnie SMK, Noel JP, Moore BS: **Algal neurotoxin biosynthesis repurposes the terpene cyclase structural fold into an *N*-prenyltransferase**. Proc Natl Acad Sci 2020, **117**:12799–12805.

Structural characterization of the *N*-geranyl-L-glutamate synthase in domoic acid biosynthesis reveals a new mechanism for a terpene cyclase-like enzyme.

18. Meda M, Kodama T, Tanaka T, Yoshizumi H, Takemoto T, Nomoto K, Fujita T: **Structures of isodomoic acids A, B and C, novel insecticidal amino acids from the red alga *Chondria armata***. *Chem Pharm Bull* 1986, **34**:4892–4895.

•19. Maeno Y, Kotaki Y, Terada R, Cho Y, Konoki K, Yotsu-Yamashita M: **Six domoic acid related compounds from the red alga, *Chondria armata*, and domoic acid biosynthesis by the diatom, *Pseudo-nitzschia multiseries***. *Sci Rep* 2018, **8**:e356.

Isolation of biosynthetic intermediates from the red algae *Chondria armata* and isotope labeling experiments in diatoms gave new insights into the biosynthesis of domoic acid.

20. Murakami S, Takemoto T, Shimizu Z: **Studies on the effective principles of *Digenea simplex* Aq. I**. *Yakugaku Zasshi* 1953, **73**:1026–1028.

21. Nitta I, Watase H, Tomiie Y: **Structure of kainic acid and its isomer, allokainic acid**. *Nature* 1958, **181**:761–762.

22. Alt A, Weiss B, Ogden AM, Knauss JL, Oler J, Ho K, Large TH, Bleakman D: **Pharmacological characterization of glutamatergic agonists and antagonists at recombinant human homomeric and heteromeric kainate receptors in vitro**. *Neuropharmacology* 2004, **46**:793–806.

23. Komiya Y, Kobayashi A: **Techniques applied in Japan for the control of *Ascaris***

and hookworm infections-A review. *Jpn J Med Sci Biol* 1965, **18**:1–17.

24. Lee SH, Kang SC, Ahn JH, Lee JW, Rim HJ: **Santonin-kainic acid complex as a mass chemotherapeutic of *Ascaris lumbricoides* control in Korea**. *Korean J Parasitol* 1972, **10**:79–85.

••25. Chekan JR, McKinnie SMK, Moore ML, Poplawski SG, Michael TP, Moore BS: **Scalable biosynthesis of the seaweed neurochemical, kainic acid**. *Angew Chem Int Ed* 2019, **58**:8454–8457.

Genome sequencing of two kainic acid producing red algae enabled discovery and in vitro validation of the biosynthetic cluster, ultimately facilitating development of a new chemoenzymatic kainic acid production route.

26. Miyasaki M, Watanabe H, Takano T, Morimoto A: **Studies on the components of *Digenea simplex* Ag. VII**. *Yakugaku Zasshi* 1956, **76**:189–191.

27. Maeno Y, Terada R, Kotaki Y, Cho Y, Konoki K, Yotsu-Yamashita M: **Possible biosynthetic products and metabolites of kainic acid from the red alga *Digenea simplex* and their biological activity**. *J Nat Prod* 2019, **82**:1627–1633.

28. Lévesque M, Avoli M: **The kainic acid model of temporal lobe epilepsy**. *Neurosci Biobehav Rev* 2013, **37**:2887–2899.

29. Stathakis CI, Yioti EG, Gallos JK: **Total syntheses of (-)-α-kainic acid**. *Eur J Org Chem* 2012, **2012**:4661–4673.

30. Wiese M, D'Agostino PM, Mihali TK, Moffitt MC, Neilan BA: **Neurotoxic alkaloids: saxitoxin and its analogs**. *Mar Drugs* 2010, **8**:2185–2211.

31. Zhang F, Xu X, Li T, Liu Z: **Shellfish toxins targeting voltage-gated sodium channels**. *Mar Drugs* 2013, **11**:4698–4723.

32. Llewellyn LE: **Saxitoxin, a toxic marine natural product that targets a multitude of receptors**. *Nat Prod Rep* 2006, **23**:200–222.

33. Turner A, Dhanji-Rapkova M, Dean K, Milligan S, Hamilton M, Thomas J, Poole C, Haycock J, Spelman-Marriott J, Watson A, et al.: **Fatal canine intoxications linked to the presence of saxitoxins in stranded marine organisms following winter storm activity**. *Toxins (Basel)* 2018, **10**:94.

34. Schantz EJ, Mold JD, Stanger DW, Shavel J, Riel FJ, Bowden JP, Lynch JM, Wyler RS, Riegel B, Sommer H: **Paralytic shellfish poison. VI. A procedure for the isolation and purification of the poison from toxic clam and mussel tissues**. *J Am Chem Soc* 1957, **79**:5230–5235.

35. Kellmann R, Mihali TK, Jeon YJ, Pickford R, Pomati F, Neilan BA: **Biosynthetic intermediate analysis and functional homology reveal a saxitoxin gene**

cluster in cyanobacteria. *Appl Environ Microbiol* 2008, **74**:4044–4053.

36. Stüken A, Orr RJS, Kellmann R, Murray SA, Neilan BA, Jakobsen KS: **Discovery of nuclear-encoded genes for the neurotoxin saxitoxin in dinoflagellates**. *PLoS One* 2011, **6:** e20096.

37. Wang H, Guo R, Lim W-A, Allen AE, Ki J-S: **Comparative transcriptomics of toxin synthesis genes between the non-toxin producing dinoflagellate *Cochlodinium polykrikoides* and toxigenic *Alexandrium pacificum***. *Harmful Algae* 2020, **93**:101777.

38. Akbar MA, Mohd Yusof NY, Tahir NI, Ahmad A, Usup G, Sahrani FK, Bunawan H: **Biosynthesis of saxitoxin in marine dinoflagellates: An omics perspective**. *Mar Drugs* 2020, **18:**e103.

39. Tsuchiya S, Cho Y, Konoki K, Nagasawa K, Oshima Y, Yotsu-Yamashita M: **Synthesis and identification of proposed biosynthetic intermediates of saxitoxin in the cyanobacterium *Anabaena circinalis* (TA04) and the dinoflagellate *Alexandrium tamarense* (Axat-2)**. *Org Biomol Chem* 2014, **12**:3016–3020.

40. Tsuchiya S, Cho Y, Yoshioka R, Konoki K, Nagasawa K, Oshima Y, Yotsu-Yamashita M: **Synthesis and identification of key biosynthetic intermediates for the formation of the tricyclic skeleton of saxitoxin**. *Angew Chemie - Int Ed* 2017, **56**:5327–5331.

41. Kellmann R, Neilan BA: **Biochemical characterization of paralytic shellfish toxin biosynthesis in vitro**. *J Phycol* 2007, **43**:497–508.

42. Shimizu Y: **Microalgal metabolites**. *Chem Rev* 1993, **93**:1685–1698.

43. Tsuchiya S, Cho Y, Konoki K, Nagasawa K, Oshima Y, Yotsu-Yamashita M: **Biosynthetic route towards saxitoxin and shunt pathway**. *Sci Rep* 2016, **6**:e20340.

••44. Chun SW, Hinze ME, Skiba MA, Narayan ARH: **Chemistry of a unique polyketide-like synthase**. *J Am Chem Soc* 2018, **140**:2430–2433.

In vitro reconstitution demonstrates that the first committed step of the saxitoxin biosynthetic pathway is catalyzed by an unusual four module polyketide-like synthase.

•45. Lukowski AL, Mallik L, Hinze ME, Carlson BM, Ellinwood DC, Pyser JB, Koutmos M, Narayan ARH: **Substrate promiscuity of a paralytic shellfish toxin amidinotransferase**. *ACS Chem Biol* 2020, **15**:626-631.

Detailed biochemical and substrate scope analysis demonstrates that an amidinotransferase catalyzes the second biosynthetic step in the production of

PSTs.

46. Lukowski AL, Ellinwood DC, Hinze ME, Deluca RJ, Du Bois J, Hall S, Narayan ARH: **C-H hydroxylation in paralytic shellfish toxin biosynthesis**. *J Am Chem Soc* 2018, **140**:11863–11869.

47. Lukowski AL, Denomme N, Hinze ME, Hall S, Isom LL, Narayan ARH: **Biocatalytic detoxification of paralytic shellfish toxins**. *ACS Chem Biol* 2019, **14:**941– 948.

• 48. Cullen A, D'Agostino PM, Mazmouz R, Pickford R, Wood S, Neilan BA: **Insertions within the saxitoxin biosynthetic gene cluster result in differential toxin profiles**. *ACS Chem Biol* 2018, **13**:3107–3114.

Differences in the saxitoxin biosynthetic genes clusters of two related cyanobacteria enabled identification and in vitro characterization of a sulfotransferase important in the conversion of saxitoxin to the suite of PSTs found in nature.

49. Lukowski AL, Liu J, Bridwell-Rabb J, Narayan ARH: **Structural basis for divergent C–H hydroxylation selectivity in two Rieske oxygenases**. *Nat Commun* 2020, **11**:2991.

50. Cho Y, Tsuchiya S, Omura T, Koike K, Oikawa H, Konoki K, Oshima Y, Yotsu-Yamashita M: **Metabolomic study of saxitoxin analogues and biosynthetic intermediates in dinoflagellates using ^{15}N-labelled sodium nitrate as a nitrogen source**. *Sci Rep* 2019, **9**:e3460.

51. Makarova M, Rycek L, Hajicek J, Baidilov D, Hudlicky T: **Tetrodotoxin: History, biology, and synthesis**. *Angew Chem Int Ed Engl* 2019, **58**:18338–18387.

52. Jal S, Khora SS: **An overview on the origin and production of tetrodotoxin, a potent neurotoxin**. *J Appl Microbiol* 2015, **119**:907–916.

53. Kono M, Matsui T, Furukawa K, Yotsu-Yamashita M, Yamamori K: **Accumulation of tetrodotoxin and 4,9-anhydrotetrodotoxin in cultured juvenile kusafugu *Fugu niphobles* by dietary administration of natural toxic komonfugu *Fugu poecilonotus* liver**. *Toxicon* 2008, **51**:1269–1273.

54. Hanifin CT, Brodie ED 3rd, Brodie ED Jr: **Tetrodotoxin levels of the rough-skin newt, *Taricha granulosa*, increase in long-term captivity**. *Toxicon* 2002, **40**:1149–1153.

55. Cardall BL, Brodie ED Jr, Brodie ED 3rd, Hanifin CT: **Secretion and regeneration of tetrodotoxin in the rough-skin newt (*Taricha granulosa*)**. *Toxicon* 2004, **44**:933–938.

56. Kudo Y, Chiba C, Konoki K, Cho Y, Yotsu-Yamashita M: **Confirmation of the absence of tetrodotoxin and its analogues in the juveniles of the Japanese**

fire-bellied newt, *Cynops pyrrhogaster*, captive-reared from eggs in the laboratory using HILIC-LC-MS. *Toxicon* 2015, **101**:101–105.

57. Lehman EM, Brodie ED Jr, Brodie ED 3rd: **No evidence for an endosymbiotic bacterial origin of tetrodotoxin in the newt *Taricha granulosa*.** *Toxicon* 2004, **44**:243–249.

• 58. Melnikova DI, Vlasenko AE, Magarlamov TY: **Stable tetrodotoxin production by *Bacillus* sp. Strain 1839.** *Mar Drugs* 2019, **17**:e704**.**

Reported discovery and maintenance of a tetrodotoxin-producing bacterial strain from a marine ribbon worm.

59. Beleneva IA, Magarlamov TY, Kukhlevsky AD: **Characterization, identification, and screening for tetrodotoxin production by bacteria associated with the ribbon worm (*Nemertea*) *Cephalotrix simula* (Ivata, 1952).** *Microbiology* 2014, **83**:220–226.

• 60. Vaelli PM, Theis KR, Williams JE, O'Connell LA, Foster JA, Eisthen HL: **The skin microbiome facilitates adaptive tetrodotoxin production in poisonous newts.** *Elife* 2020, **9:**e53898.

First report of bacterial strains with measurable tetrodotoxin isolated from a terrestrial organism.

61. Magarlamov TY, Melnikova DI, Chernyshev AV: **Tetrodotoxin-producing bacteria: Detection, distribution and migration of the toxin in aquatic systems.** *Toxins* 2017, **9**:e166.

62. Azevedo GPR, da Paz PHC, Mattsson HK, Moreira APB, Leomil L, Calegário G, Appolinario L, Vidal L, Silva BS, Chimetto Tonon LA, et al.: **Genome sequence of *Shewanella corallii* strain A687 isolated from pufferfish (*Sphoeroides spengleri*).** *Genet Mol Biol* 2020, **43:**e20180314.

63. Kudo Y, Yamashita Y, Mebs D, Cho Y, Konoki K, Yasumoto T, Yotsu-Yamashita M: **C5-C10 directly bonded tetrodotoxin analogues: Possible biosynthetic precursors of tetrodotoxin from newts.** *Angew Chem Int Ed* 2014, **53**:14546–14549.

64. Kudo Y, Yasumoto T, Mebs D, Cho Y, Konoki K, Yotsu-Yamashita M: **Cyclic guanidine compounds from toxic newts support the hypothesis that tetrodotoxin is derived from a monoterpene.** *Angew Chem Int Ed Engl* 2016, **55**:8728–8731.

•• 65. Kudo Y, Yotsu-Yamashita M: **Isolation and biological activity of 8-epitetrodotoxin and the structure of a possible biosynthetic shunt product of tetrodotoxin, Cep-226A, from the newt *Cynops ensicauda popei*.** *J Nat Prod* 2019, **82**:1656–1663.

The most recent report (along with references 63 and 64) and interpretation of tetrodotoxin co-accumulated compounds in newts suggestive of a monoterpene biosynthetic origin.

• 66. Kudo Y, Chiba C, Konoki K, Cho Y, Yotsu-Yamashita M: **Dietary administration of tetrodotoxin and its putative biosynthetic intermediates to the captive-reared non-toxic Japanese fire-bellied newt, *Cynops pyrrhogaster***. *Toxicon* 2017, **137**:78–82.

Key feeding experiments of candidate biosynthetic intermediates to non-toxic newts.

•• 67. Ueyama N, Sugimoto K, Kudo Y, Onodera K-I, Cho Y, Konoki K, Nishikawa T, Yotsu-Yamashita M: **Spiro bicyclic guanidino compounds from pufferfish: Possible biosynthetic intermediates of tetrodotoxin in marine environments**. *Chemistry* 2018, **24**:7250–7258.

The most recent report and interpretation of tetrodotoxin co-accumulated compounds in marine organisms (pufferfish).

68. Yotsu-Yamashita M, Yamagishi Y, Yasumoto T: **5,6,11-Trideoxytetrodotoxin from the puffer fish, *Fugu poecilonotus***. *Tetrahedron Lett* 1995, **36**:9329–9332.

69. Yotsu-Yamashita M, Abe Y, Kudo Y, Ritson-Williams R, Paul VJ, Konoki K, Cho Y, Adachi M, Imazu T, Nishikawa T, Isobe M: **First identification of 5,11-dideoxytetrodotoxin in marine animals, and characterization of major fragment ions of tetrodotoxin and its analogs by high resolution ESI-MS/MS**. *Marine Drugs* 2013 **11**:2799–2813.

70. Nicolaou KC, Frederick MO, Aversa RJ: **The continuing saga of the marine polyether biotoxins**. *Angew Chem Int Ed Engl* 2008, **47**:7182–7225.

71. Van Dolah FM, Kohli GS, Morey JS, Murray SA: **Both modular and single-domain type I polyketide synthases are expressed in the brevetoxin-producing dinoflagellate, *Karenia brevis* (Dinophyceae)**. *J Phycol* 2017, **53**:1325–1339.

72. Kretzschmar AL: **Delving into the genetic code of *Gambierdiscus* - The devil is in the detail [PhD thesis].** University of Technology Sydney, 2019

73. Verma A, Kohli GS, Harwood DT, Ralph PJ, Murray SA: **Transcriptomic investigation into polyketide toxin synthesis in *Ostreopsis* (Dinophyceae) species**. *Environ Microbiol* 2019, **21**:4196–4211.

74. Verma A, Barua A, Ruvindy R, Savela H, Ajani PA, Murray SA: **The genetic basis of toxin biosynthesis in dinoflagellates**. *Microorganisms* 2019, **7**:e222.

75. Wan X, Yao G, Liu Y, Chen J, Jiang H: **Research progress in the biosynthetic**

mechanisms of marine polyether toxins. *Mar Drugs* 2019, **17**:e594.

76. Manning SR, La Claire JW: **Prymnesins: toxic metabolites of the golden alga, *Prymnesium parvum* Carter (Haptophyta)**. *Mar Drugs* 2010, **8**:678–704.

77. Rasmussen SA, Meier S, Andersen NG, Blossom HE, Duus JØ, Nielsen KF, Hansen PJ, Larsen TO: **Chemodiversity of ladder-frame prymnesin polyethers in *Prymnesium parvum*.** *J Nat Prod* 2016, **79**:2250–2256.

78. Lee MS, Repeta DJ, Nakanishi K, Zagorski MG: **Biosynthetic origins and assignments of carbon 13 NMR peaks of brevetoxin B**. *J Am Chem Soc* 1986, **108**:7855–7856.

79. Snyder RV, Guerrero MA, Sinigalliano CD, Winshell J, Perez R, Lopez JV, Rein KS: **Localization of polyketide synthase encoding genes to the toxic dinoflagellate *Karenia brevis***. *Phytochemistry* 2005, **66**:1767–1780.

80. Van Wagoner RM, Satake M, Wright JLC: **Polyketide biosynthesis in dinoflagellates: What makes it different?** *Nat Prod Rep* 2014, **31**:1101–1137.

81. Gallimore AR, Spencer JB: **Stereochemical uniformity in marine polyether ladders—Implications for the biosynthesis and structure of maitotoxin.** *Angew Chem Int Ed* 2006, **45:**4406–4413.

82. Nakanishi K: **The chemistry of brevetoxins: A review**. *Toxicon* 1985, **23**:473–479.

83. Vilotijevic I, Jamison TF. **Epoxide-opening cascades in the synthesis of polycyclic polyether natural products.** *Angew Chem Int Ed* 2009 **48**:5250–5281.

84. Anttila M, Strangman W, York R, Tomas C, Wright JLC: **Biosynthetic studies of 13-desmethylspirolide C produced by *Alexandrium ostenfeldii* (= *A. peruvianum*): Rationalization of the biosynthetic pathway following incorporation of ^{13}C-labeled methionine and application of the odd–even rule of methylation**. *J Nat Prod* 2016, **79**:484–489.

85. Riaz S, Sui Z, Niaz Z, Khan S, Liu Y, Liu H: **Distinctive nuclear features of dinoflagellates with a particular focus on histone and histone-replacement proteins**. *Microorganisms* 2018, **6:**e128.

86. Wisecaver JH, Hackett JD: **Dinoflagellate genome evolution**. *Annu Rev Microbiol* 2011, **65**:369–387.

87. Haq S, Bachvaroff TR, Place AR: **Characterization of acetyl-CoA carboxylases in the basal dinoflagellate *Amphidinium carterae***. *Mar Drugs* 2017, **15:**e149.

• 88. Van Dolah FM, Morey JS, Milne S, Ung A, Anderson PE, Chinain M: **Transcriptomic analysis of polyketide synthases in a highly ciguatoxic**

dinoflagellate, *Gambierdiscus polynesiensis* and low toxicity *Gambierdiscus pacificus*, from French Polynesia. *PloS One* 2020, **15**:e0231400.

The most recent report and interpretation of the *de novo* transcriptomic approach to dinoflagellate polyether biosynthesis.