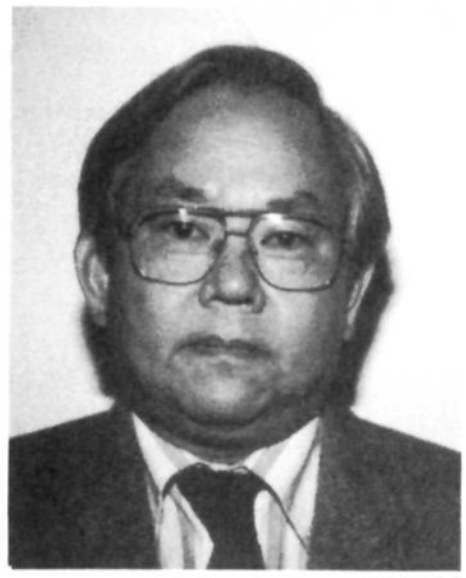
Microalgal Metabolites

Yuzuru Shimizu

Department of Pharmacognosy and Environmental Health Sciences, College of Pharmacy, The University of Rhode Island, Kingston, Rhode Island 02881

Received December 8, 1992 (Revised Manuscript Received February 26, 1993)

Contents



Yuzuru Shimizu was born in Gifu, Japan, completed his B.S., M.S., and Ph.D. in pharmaceutical Sciences at Hokkaido University, Sapporo, Japan. He did his postdoctoral work at Worcester Foundation for Experimental Biology in 1963–1964, and at University of Georgia, in 1965. He was assistant professor from 1965–1969 at Faculty of Pharmaceutical Sciences, Hokkaido University. He moved to the University of Rhode Island in 1969. There, he is currently Professor of Pharmacognosy and Chemistry and Chairman of the Department of Pharmacognosy and Environmental Health Sciences. He was a past president of American Society of Pharmacognosy, and was chairman of Gordon Research Conference, Marine Natural Products Chemistry. His research interest is in marine natural products chemistry, especially marine toxins and microalgal chemistry.

I. Introduction

Microalgae play an important role in the marine biological system. With their photosynthetic ability, they are the major producer of biomass and organic compounds in the oceans. Many algal metabolites have unique structures and are formed by biosynthetic routes quite different from those known for terrestrial metabolites. This review is intended to look at the secondary metabolites of marine microalgae from the view point of biosynthesis and their roles in the biogenesis of metabolites found in other marine organisms. Since many of the algal metabolites are discussed by other authors in this issue,[1–3] the description of their chemicophysical and pharmacological characteristics of the compounds will be avoided. Also, biosynthetically uncharacteristic metabolites and the normal cell constituents such as sterols, lipids, and pigments are excluded from this article. The subject will be limited to marine microalgae in accordance with the theme of this issue, although there is no clear scientific distinction between marine and freshwater microalgae.

II. Characteristics of Marine Microalgae and Metabolism

Although the traditional sources of secondary metabolites were terrestrial higher plants, animals, and microorganisms, marine organisms have been the major targets for natural products research in the past decade. A variety of compounds with unique chemical structures and biological activity have been discovered in marine organisms, especially, in marine invertebrates.[4] However, there have been persisting questions regarding their true origins or progenitors of these metabolites. It is a widespread belief that many of them are not primary metabolites of the organisms where the compounds were found, but originated elsewhere. The possible primary producers of the secondary metabolites are microalgae, bacteria, and fungi, and they are carried through symbiosis, association, food chain, and other forms of nutrient dependency (Figure 1). The ocean is a very complex ecosystem, where all organisms live in mutual dependency. There is a persistent theory that even in what is considered to be a pure species, an endosymbiotic relationship can exist, and certain metabolites may be formed only by the collaboration of two or more organisms. Thus there is a possibility that what we consider microalgal metabolites may actually be derived from bacteria or other microorganisms associated with the algae. It should be also noted that many of the microscopic algae, which are suspected to produce interesting metabolites, have not been cultured under axenic conditions, and little is

0009-2665/93/0793-1685$12.00/0

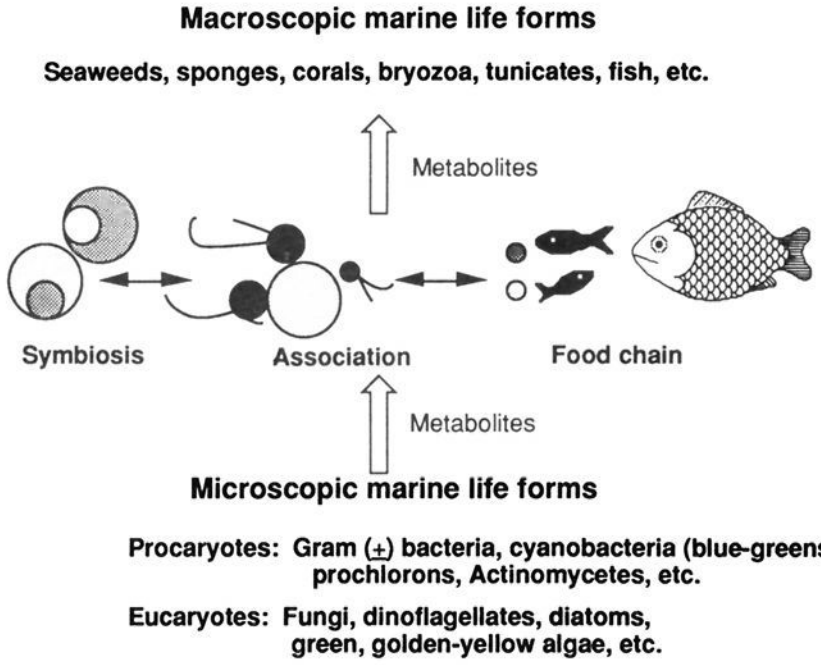


Figure 1. Transfer of metabolites in marine ecosystems.

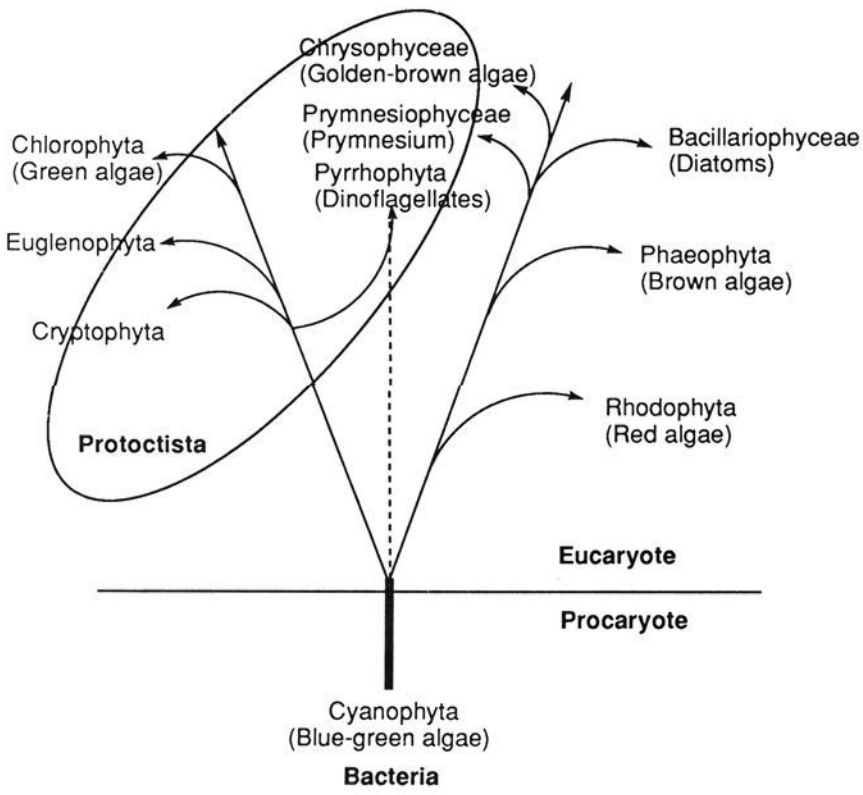


Figure 2. Approximate phylogenic relationship of microalgae.

known about their true ability to produce specific metabolites as single species.

Marine microalgae compose the majority of living species found in the oceans. There is no definite estimate of the total number of the existing species. New species are being discovered constantly, and the number is ever increasing. Currently, more than 10000 known species are divided into five major divisions of marine microalgae: Chlorophyta (green algae), Chrysophyta (golden-brown, yellow algae, and diatoms), Pyrrhophyta (dinoflagellates), Eugelnophyta, and Cyanophyta (blue-green algae).

The phylogenic positions and physiologic characteristics of the organisms are important to consider in studying their metabolism and biochemistry. Figure 2 shows the approximate positions of major organisms discussed in this article. However, the taxonomy and phylogenic relationship of microalgae are the subjects on which taxonomists have never agreed.[5] Thus, there are many discrepancies in the names of species and their positions on the taxonomic map, causing considerable confusions in chemical literatures.

One important issue is the handling of Cyanophyta, "blue-green algae". They are customarily called blue-green algae or simply blue-greens. However, strict disciplinarians place them in bacteria (cyanobacteria) and refuse to include them in the category of algae, because of their procaryotic nature. Nonetheless, the organisms are photosynthetic and share many algal characteristics with the eucaryotic counterparts. Moreover, it is generally believed that most photosynthetic algae (or at least their chloroplasts) have their phylogenic origin in Cyanophyta. Therefore, this author has decided to include them in this review.

Another pending issue in the taxonomy of microalgae is whether nonphotosynthetic organisms should be included into the plant kingdom or not. For example, a great number of dinoflagellates lack chlorophylls and live on heterotrophy. Thus, they are also classified as Protozoa in the animal kingdom. On the other hand, euglenoids, despite their photosynthetic ability, are traditionally included in the animal kingdom. An attempt to categorize those organisms simply by the presence or absence of photosynthetic ability is probably of little significance, because we can find both photosynthetic and heterotrophic strains among many closely related organisms or even within the same species. In fact, many photosynthetic dinoflagellates are known to participate in heterotrophy depending upon the environmental conditions. Recently, the kingdom Protoctista has been proposed to cover all organisms which are neither animal nor plant.[6] They include the majority of microalgae covered in this text. It is expected that this classification will become more common in the future.

III. Metabolites of Dinoflagellates

Dinoflagellates are flagellated organisms, both photosynthetic and heterotrophic. More than 4000 species are known to date.[7] Many fossil dinoflagellates are also known and are considered to be one of the source organisms of petroleum. The phylogenic position of dinoflagellates is unique and is assumed to be at the border of procaryotes and eucaryotes. Thus, some people have proposed to call them "mesocaryotes".[8] The living style of the organisms is diverse, and many of them occupy symbiotic environments. Some dinoflagellates are bioluminscent, and they are probably the most important contributors to the luminescence in the oceans.

The chemistry of dinoflagellates has been focused on their toxin production and pigment compositions, and only a small number of the organisms have been investigated for other secondary metabolites. Table I shows representative secondary metabolites from dinoflagellates.

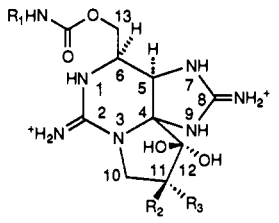
A. Saxitoxin Derivatives

Saxitoxin (1, Chart I) was first recognized as the toxic principle of so-called paralytic shellfish poisoning (PSP). It is the first marine compound whose origin was traced to microscopic plankton.[9] More recently, more than a dozen compounds have been isolated from such organisms as *Alexandrium* (formerly *Gonyaulax* or *Protogonyaulax*), *Gymnodinium*, and *Pyrodinium*

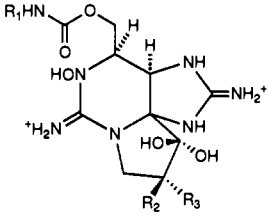
Table I. Marine Dinoflagellates Known To Produce Secondary Metabolites

organisms	type of metabolites
Amphidinium spp.	macrolides: amphidinolides
Alexandrium tamarense	heterocycles: saxitoxin, gonyautoxin derivatives
A. catenellum	
A. acatenella	
Pyrodinium bahamense var. *compressa*	
Gymnodinium catenatum	
Dinophysis spp.	polyethers: okadaic acid derivatives macrolides
Gambierdiscus toxicus	polycyclic ethers: ciguatoxin derivatives
Goniodoma (*Alexadrium*) spp.	macrolides: gonyodomin
Gymnodinium breve	polycyclic ethers: brevetoxins, hemibrevetoxins
Ostreopsis spp.	polyethers?
Prorocentrum concavum	polyethers: okadaic acid, macrolides
P. lima	

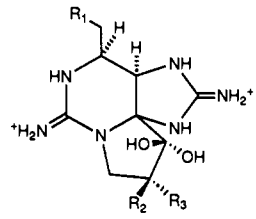
Chart I



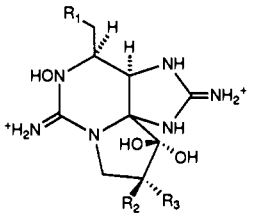
1. R_1, R_2, R_3=H
4. R_1, R_2=H, R_3=OSO_3^-
5. R_1, R_3=H, R_2=OSO_3^-
7. R_1=SO_3^-, R_2, R_3=H
9. R_1=SO_3^-, R_2=H, R_3=OSO_3^-
10. R_1=SO_3^-, R_2=OSO_3^-, R_3=H



2. R_1, R_2, R_3=H
3. R_1, R_2=H, R_3=OSO_3^-
6. R_1, R_3=H, R_2=OSO_3^-
8. R_1=SO_3^-, R_2, R_3=H
11. R_1=SO_3^-, R_2=H, R_3=OSO_3^-
12.. R_1=SO_3^-, R_2=OSO_3^-, R_3=H



13. R_1=OH, R_2=H; R_3=OSO_3^-
14. R_1=OH, R_3=H; R_2=OSO_3^-
16. R_1=H, R_2=H, R_3=H
17. R_1=H, R_2=H; R_3=OSO_3^-
18. R_1=H, R_3=H; R_2=OSO_3^-

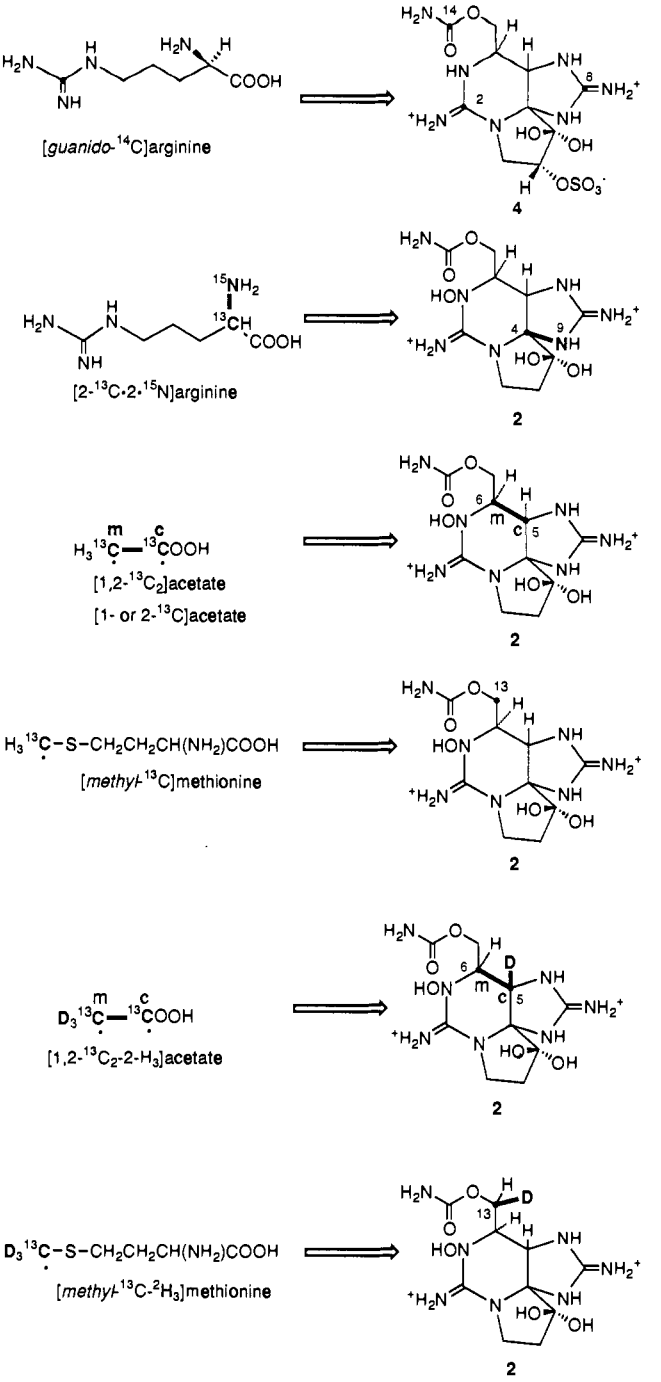


15. R_1=OH, R_2, R_3=H

spp.[10-13] Neosaxitoxin and saxitoxin are also produced by the fresh-water blue-green, *Aphanizomenon flos-aquae*.[14-16]

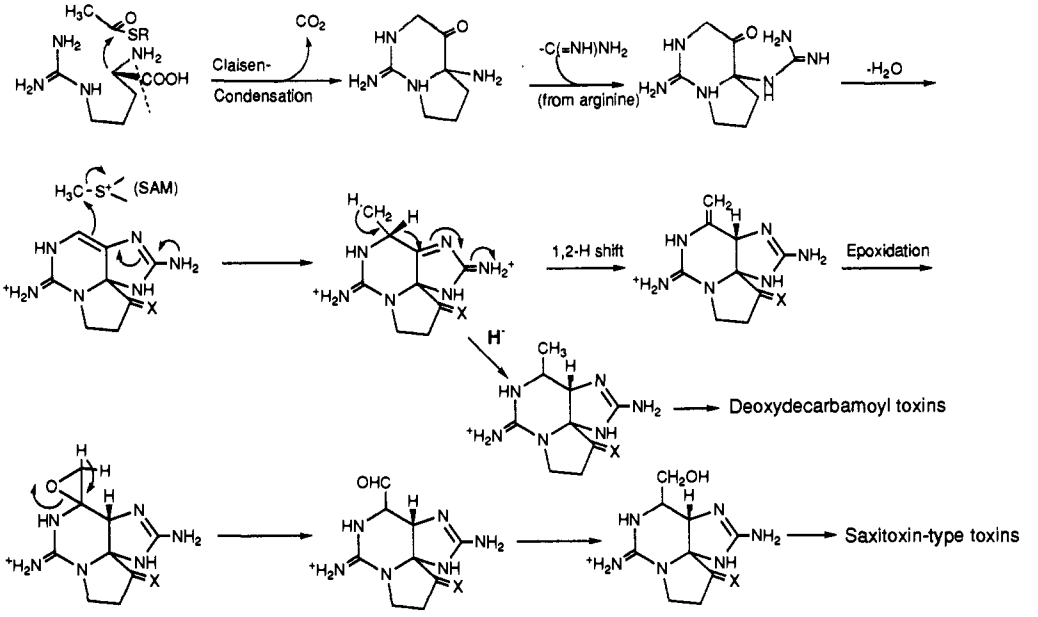
Structurally, the toxins can be divided into two major groups: saxitoxin (1) and neosaxitoxin (2, Chart I). The compounds in both groups are further diversified by the presence of 11-*O*-sulfate (3–6) or *N*-sulfate (7–12) group, the absence of carbamoyl groups (13–15), and oxygen at C-13 (16 and 18).[17] Structural studies and the chemistry of the compounds were previously reviewed by this author.[10,11]

Scheme I. Incorporation Patterns of Labeled Precursors into Saxitoxin Derivatives (Solid Lines Denote the Isotope Connectivity Shown by NMR)



The biosynthesis of these compounds has been extensively studied using labeled precursors with *Alexandrium tamarense* and *Aphanizomenon flos-aquae* (Scheme I). It was first determined from gonyautoxin-II (3) that the carbamoyl group and two guanidinium groups are derived from the guanido group of arginine.[18] The perhydropurine skeleton is not a product of the ordinary purine metabolism as might be perceived. Instead, the skeleton of the tricyclic ring system is formed by the Claisen-type condensation of acetate on the α-carbon of arginine with the loss of the carboxyl group of arginine and subsequent amidation and cyclization.[19] The side-chain carbon is derived from methionine via *S*-adenosylmethionine (SAM). Thus, the feeding of [2-^{13}C]acetate and [1,2-$^{13}C_2$]acetate

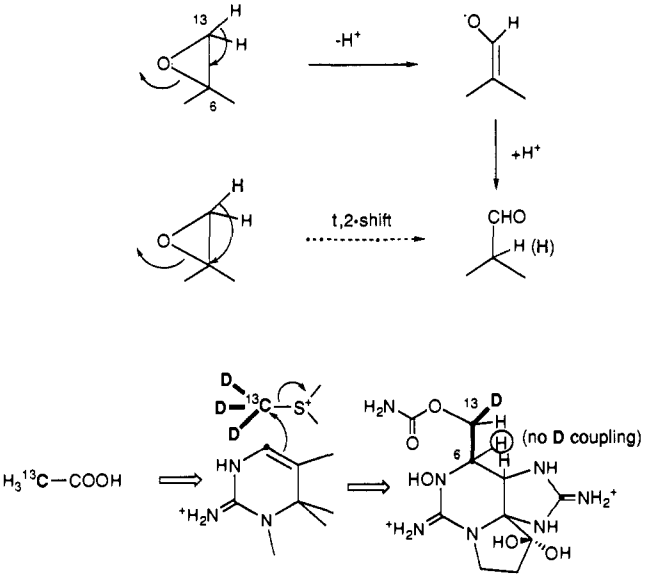
Scheme II. A Proposed Biosynthetic Pathway of Saxitoxin Derivatives[21]



showed that C-5 and C-6 come from one acetate unit, and the rest of the ring system is derived from arginine. When [2-^{13}C-2-^{15}N]arginine was fed to the organism, the connectivity of ^{13}C–^{15}N was incorporated intact in the product. By feeding [2-^{13}C-2-H_3]acetate, it was established that the hydrogen found at C-6 is not from acetate group and that a hydrogen on the acetate methyl group rearranges to C-5, probably in the course of methylation. It was further demonstrated by feeding [*methyl*-^{13}C,$^{2}H_3$]methionine that only one of the methionine methyl hydrogens is left on the side-chain methylene group. On the basis of these results, it was proposed that the side-chain carbon is introduced by electrophilic attack on the double bond followed by the migration of a hydride ion and elimination of a proton. The conversion of the resulting terminal methylene group to the carbinol may proceed through epoxide formation followed by opening to an aldehyde and reduction.[20] Such a process explains the retention of only one deuterium atom in the product (Scheme II).[21] The recently found group of deoxydecabamoyl toxins[17] can be formed by the attack of hydride ion on C-6 carbonium ion instead of proton elimination from C-13 methyl group. It was also confirmed by the stepwise feeding of [2-^{13}C]acetate and [*methyl*-^{13}C,$^{2}H_3$]-methionine that there is no rearrangement of a hydrogen from methyl group to C-6 position.[22] These results allude to a rather unprecedented biosynthetic pathway of saxitoxin derivatives (Scheme III).[22]

The presence of sulfate conjugation is another characteristic feature of these toxins. In fact, most toxins occur as 11-*O*-sulfate and/or *N*-sulfocarbamoyl derivatives in the dinoflagellates, and saxitoxin is a minor component. The occurrence of *N*-sulfate groups is rather rare among natural products. Only a few cases of *N*-sulfated derivatives are known in β-lactams and aminopolysaccharides, but an *N*-sulfocarbamoyl group has not reported previously. These *N*-sulfate groups are easily hydrolyzed by weak acids,[23] and also possibly by *N*-sulfatase in the biological system.[24] It is not clear if the formation of the sulfated toxins precedes the unsulfated compounds in the dinoflagellates. However, it was proved that the reductive cleavage of *O*-sulfate

Scheme III. The Origin of H-6 and the Result of Stepwise Feeding of [2-^{13}C]Acetate and [*methyl*-^{13}C,$^{2}H_3$]Methionine in the Biosynthesis of Saxitoxin Derivatives (Solid Lines Denote the Presence of Isotope Connectivity Shown by NMR)[21]



could take place in shellfish to give unsulfated toxins such as saxitoxin.[25,26] Similarly, the *N*-hydroxy group of neosaxitoxin series can be reductively removed. Again, it is not known if *N*-hydroxy derivatives are the precursors of saxitoxin type compounds or the other way around in the dinoflagellates (Scheme IV).

It should be noted that three molecules of arginine are required to build the nitrogen-rich molecule (Figure 3). This may be highly significant to the organism with respect to its nitrogen metabolism, because, in some strains, the toxin levels reach 60 pg/cell, which represents an enormous portion of the total organic content of a small cell (~20-μm diameter). This occurrence of a high concentration of amino acid metabolites may be interpreted as a mechanism to treat excess amino acids in the cells. The biological significance of these compounds is often discussed in terms of self-defense.

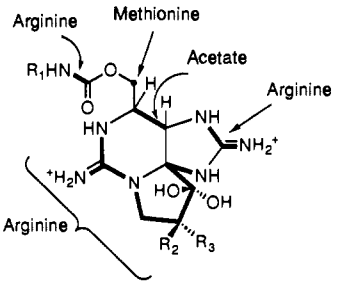
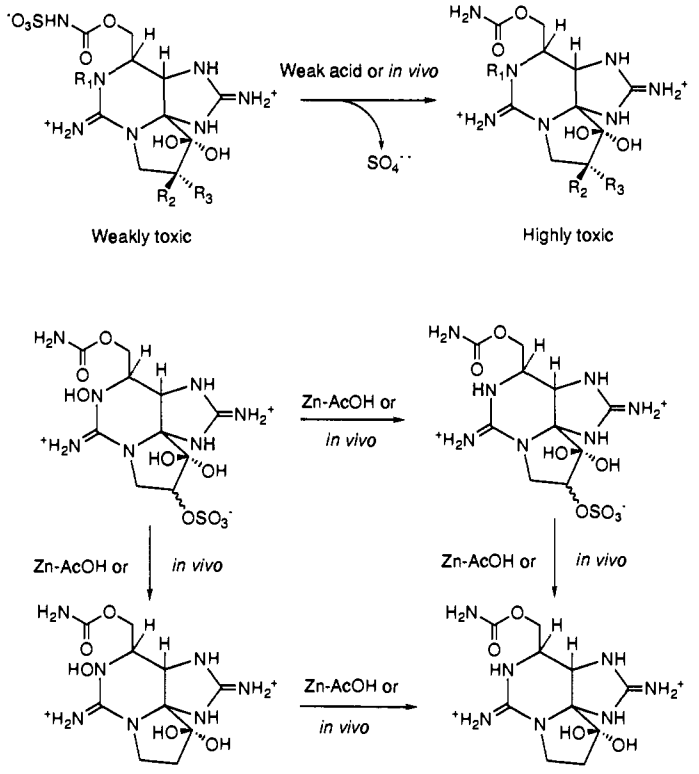


Figure 3. Biosynthetic building blocks of saxitoxin derivatives.

Scheme IV. Chemical and Bioconversion of Saxitoxin Derivatives

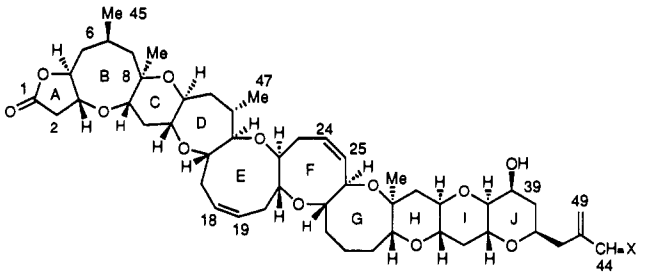


However, the toxigenicity of the organism varies from strain to strain even within the same species with no apparent effect on their chance of survival.

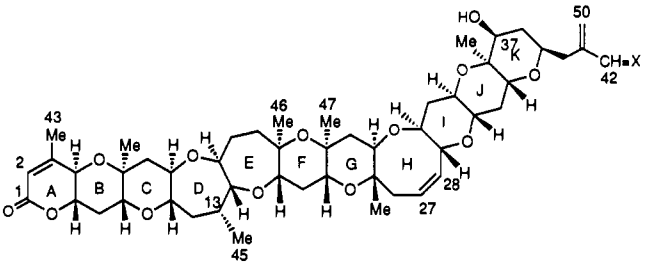
B. Polycyclic Ether Type Metabolites

One of the most characteristic and spectacular classes of compounds produced by dinoflagellates is the polycyclic ethers. The linear-condensed structure was first demonstrated in brevetoxin B (= GB-2 toxin or PBTX-2) (**19**). The compound, which is the major toxin in the Florida red tide organism, *Gymnodinium breve*, had been investigated under different names by several groups,[27-29] but its unprecedented structure was established by X-ray crystallography in 1980.[30] Subsequently, the structure of the most toxic component, brevetoxin A (**20**, Chart II) was elucidated by X-ray crystallography.[31] While several other toxins isolated from the organism were divided into brevetoxin A series (**21** and **22**) and brevetoxin B series (**23–25**) by their skeletons, a new type of toxin, hemibrevetoxins (**26**),[32] which have about a half-size skeleton of brevetoxins, has been recently discovered in the same organism.

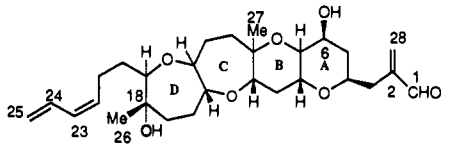
Chart II



20 X=O: brevetoxin A (GB-1)
21 X=H, OH: GB-7
22 X=$(OCH_3)_2$



19 X=O: brevetoxin B (GB-2)
23 X=H, OH: GB-3
24 X=O, 37-OAc: GB-5
25 X=O, 27, 28β-epoxide: GB-6



26 Hemibrevetoxin B

The biosynthesis of brevetoxins has been a subject of speculation as soon as the structure of brevetoxin B was revealed. The *all-trans* cyclic structure can be formed by a cascade of opening of all-*trans* epoxides, which are probably formed by epoxidation of *trans*-double bonds (Figure 4).[33,34] The consecutive opening of the epoxides could start from either side of the molecule, but the structure of hemibrevetoxin B (**26**), which represents the right half of brevetoxin structure

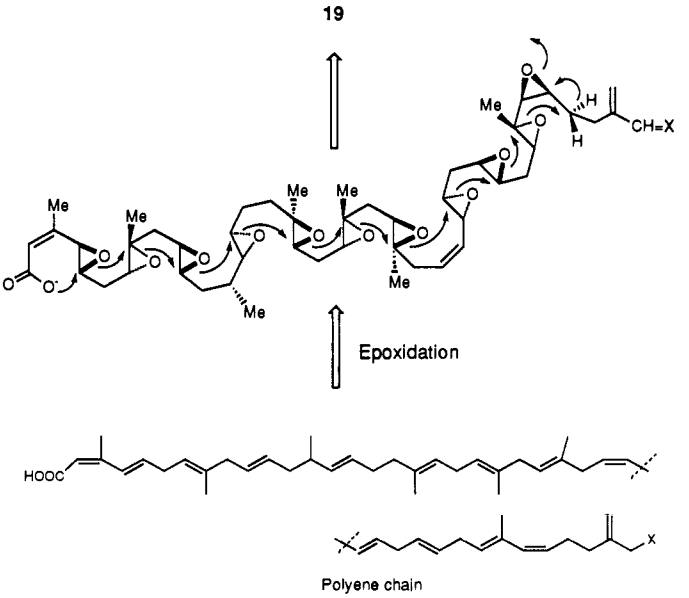


Figure 4. Polyene–epoxide mechanism of polycyclic ether formation in brevetoxin biosynthesis.

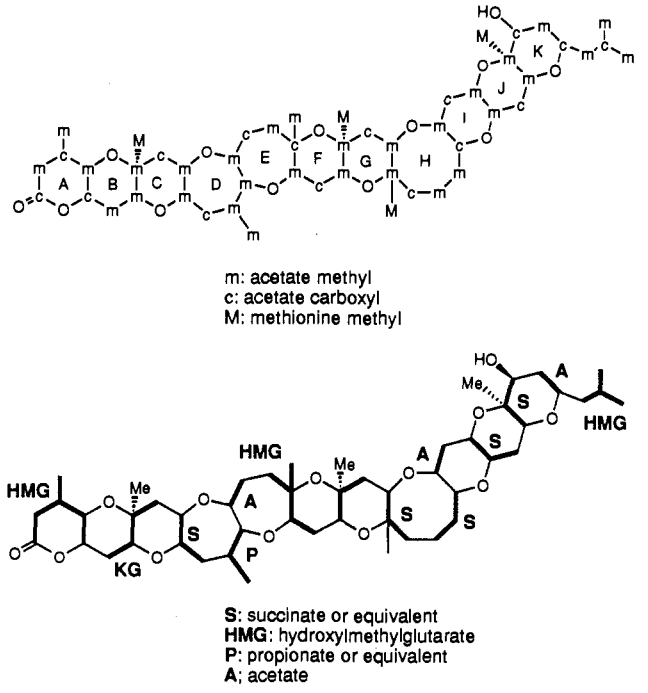
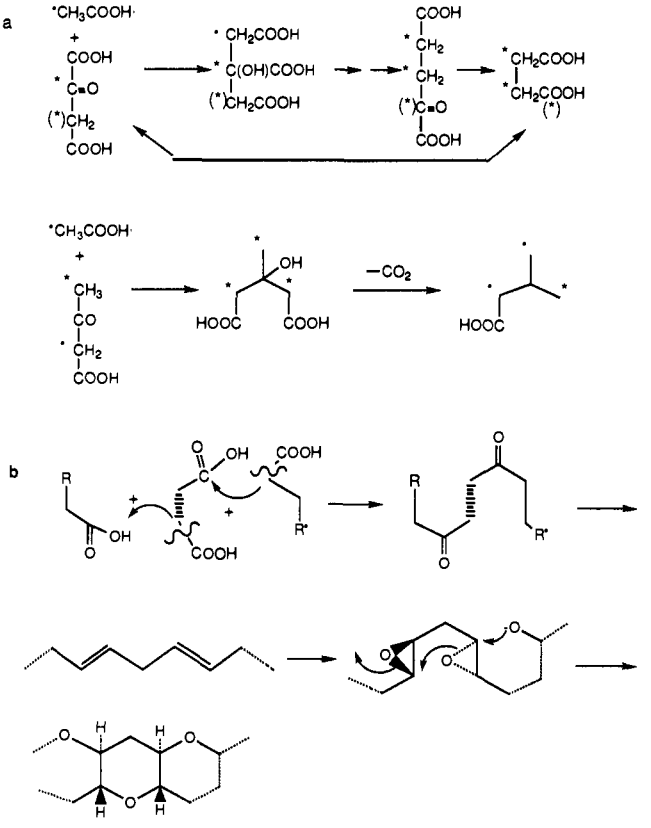


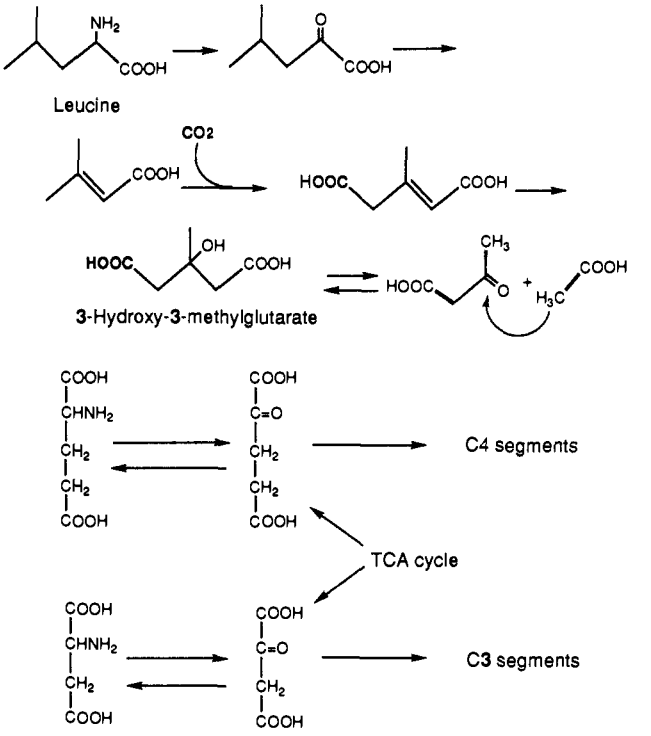
Figure 5. Incorporation patterns of labeled acetate into brevetoxin B (19), and hypothetical building blocks of the molecule.[36]

Scheme V. The Fate of Acetate Labels in Putative Building Blocks (a) and the Possible Mechanism of Carbon Chain Formation in the Biosynthesis of Brevetoxins and Other Polyether Compounds in Dinoflagellates (b)[36]

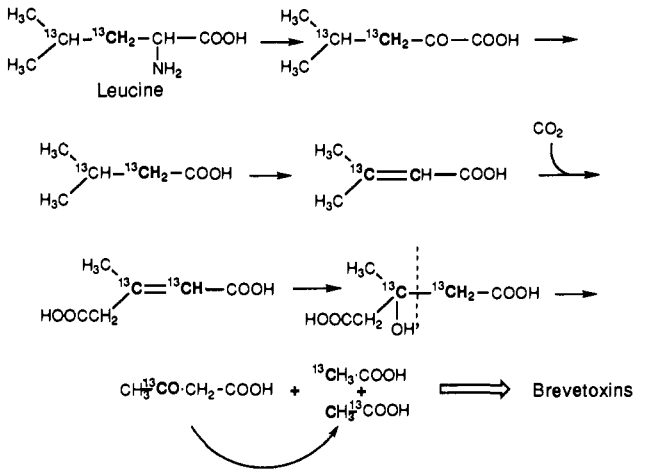


strongly indicates that the cyclization starts from the right-hand side of the molecule, probably by the opening of *cis*-epoxide and hydride ion shift (Figure 4). The sequence is very similar to the mechanism proposed

Scheme VI. Hypothetical Alternative Pathways to HMGA, α-Ketoglutarate and Succinate in Dinoflagellates



Scheme VII. Possible Fate of Exogenous Leucine Fed to *Gymnodinium breve* as Judged from the Labeling Pattern in Brevetoxins[37]



for the biosynthesis of polyether antibiotics. Thus, one may simply assume that as in the case of polyether antibiotics, the basic polyene carbon chain of brevetoxin is also an acetogenin biosynthesized from acetate units, and the methyl branches are introduced by substituting acetate with propionate or from methionine by methylation with *S*-adenosylmethionine. The feeding experiments with labeled acetate, however, gave labeling patterns which could not be interpreted by the simple acetogenin pathway (Figure 5).[35,36] It was concluded from the pulse feeding of [2-^{13}C]acetate that labeled acetate was metabolized through the TCA cycle and incorporated into dicarboxylic acids before utilized for the toxin biosynthesis (Scheme V).[36] The result strongly implicates a new type of biosynthesis, in which an acetogenin-like carbon chain is formed from C_4 and C_5 dicarboxylic acids by Claisen-type condensation at both

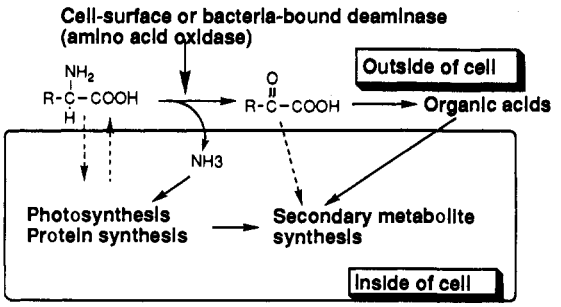


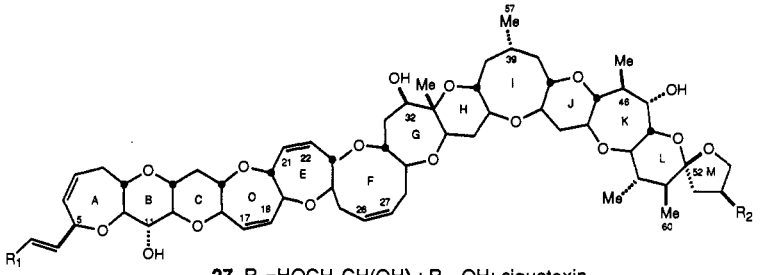
Figure 6. Possible carbon recycling and utilization of exogenous amino acids by *Gymnodinium breve*.

ends of the molecules followed by decarboxylation. It is drastically different from the known polyketide biosynthesis, which involves chain formation by the condensation of acetate (malonate) or propionate (methylmalonate) at their α-position.

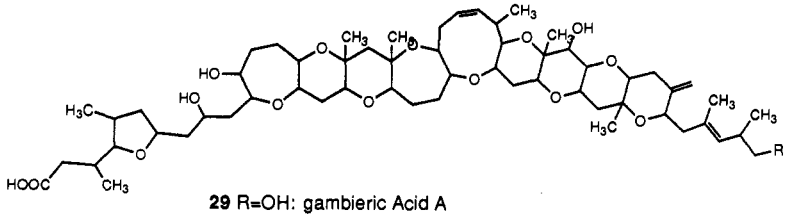
Experiments to feed dicarboxylic acids as putative precursors have been so far unsuccessful. Recently, there is new speculation that some of the building blocks might be derived from unconventional origins, i.e. amino acids such as aspartate, glutamate, and leucine.[37] In fact, some strange labeling patterns observed in the past feeding experiments[38] can be explained by this hypothesis. For example, 3-hydroxy-3-methylglutarate can come from the degradation of leucine in addition to the normal pathway, which involves the condensation of three acetate units (Scheme VI). However, the feeding attempts with various labeled amino acid precursors have so far been unproductive, because of the dinoflagellate's refusal to incorporate exogenous organic compounds. So far the only molecule which has been found to reach the biosynthetic site in intact form is acetate. The other compounds tried were either completely rejected or incorporated only after being metabolized to acetate. In the case of feeding experiments with [3-^{13}C]- and [3,4-^{13}C]leucine, leucine was degraded to acetate before being utilized for the toxin biosynthesis (Scheme VII).[37] These biodegradations probably occur outside of the cell by cell surface enzymes and the associated bacteria. This recycling of organic compounds (called "carbon loop") seems to be common in marine phytoplankton and may be important to consider the biosynthesis of microalgal metabolites (Figure 6).

After brevetoxins were shown to have unprecedented polycyclic ether structures, more compounds were found to have similar skeletons. The most important of them is ciguatoxin (**27**), which was isolated as the toxic principal of ciguatoxic moray eels.[39,40] It was proposed that the compound is produced by the dinoflagellate, *Gambierdiscus toxicus* and accumulated in the eel through the food chain. In fact, Yasumoto's group showed that the wild cells of *G. toxicus* contain closely related compounds such as GT4b (**28**), which could be the precursors of ciguatoxin (**27**, Chart III). Several other polyether compounds, e.g. gambieric acids A (**29**) and C (**30**) have also been found in various strains of *G. toxicus*.[41] It is very likely that these metabolites are also biosynthesized by a mechanism similar to that of the brevetoxins. Another toxin produced by *G. toxicus*, maitotoxin, is a potent Ca^{2+} ion channel activator and has been proposed to have a partial structure, **31**, of a

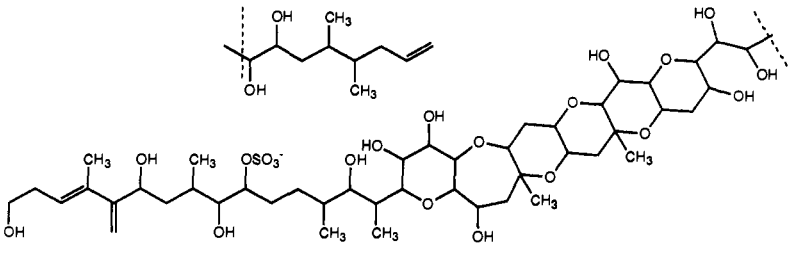
Chart III



27 R_1=$HOCH_2CH(OH)$-; R_2=OH: ciguatoxin
28 R_1= CH_2=CH-; R_2=H: GT4b



29 R=OH: gambieric Acid A
30 R= : gambieric Acid C



31 Maitotoxin (partial structure)

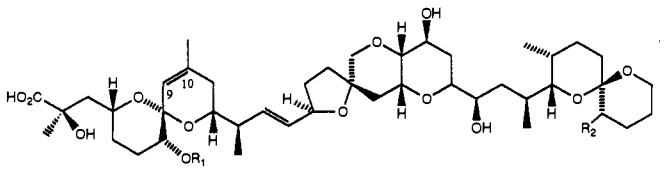
long alkyl chain linked with polycyclic ether and polyhydroxy moieties.[42] It indicates that the carbon chains of the polycyclic ether compounds and the long-chain alkyl compounds found in dinoflagellates including macrolides discussed below have the same biogenetic origin. The structural differences seem to arise from the different modes of epoxide opening.

C. Macrolides and Oxygenated Acyclic Compounds

Dinoflagellates produce polyether compounds and macrolide compounds which are very similar to the macrolide antibiotics produced by *Streptomyces*. The former group is represented by okadaic acid (**32**, Chart IV), a potent protein phosphatase inhibitor.[43,44] The latter includes a series of cytotoxic compounds, amphidinolides (**33–37**, Chart V), which are produced by *Amphidinium* sp.,[45–48] prorocentrolide (**38**) from *Prorocentrium lima*,[49] and goniodomin A (**39**, Chart VI), an antifungal agent from *Goniodoma* (*Gonyaulax*) sp.[50]

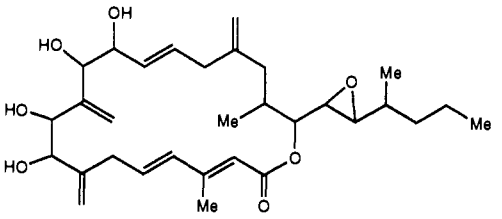
Okadaic acid (**32**) was first isolated from the sponge, *Halichondria okadaii*,[51] but it was isolated from the cultured dinoflagellates, *P. lima*[52] and *P. concavum*,[53] and also from the wild cells of *Dinophysis* spp.[54] This group also includes acanthifolicin (**40**) isolated from the sponge, *Pandoras acanthifolium*,[55] and dinophysistoxin-1 (**41**) and dinophysistoxin-3 (**42**) from toxic shellfish.[56] It is not known if some of these compounds are the primary products of dinoflagellates or those structurally modified after entering into the bodies of the invertebrates. However, dinophysistoxin-1 (**41**), which is a homolog of okadaic acid, was identified as a metabolite in the wild cells of *Dinophysis* spp. and cultured *Prorocentrum lima*.[57]

Chart IV

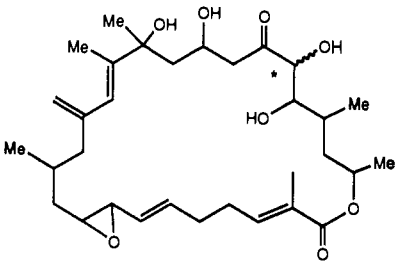


32 R_1=H, R_2=H: okadaic acid
40 R_1=H, R_2=H, 9,10-α-episulfide: acanthifolicin;
41 R_1=H, R_2=CH_3:dinophysistoxin-1
42 R_1=Acyl, R_2=CH_3: dinophysistoxin-3

Chart V

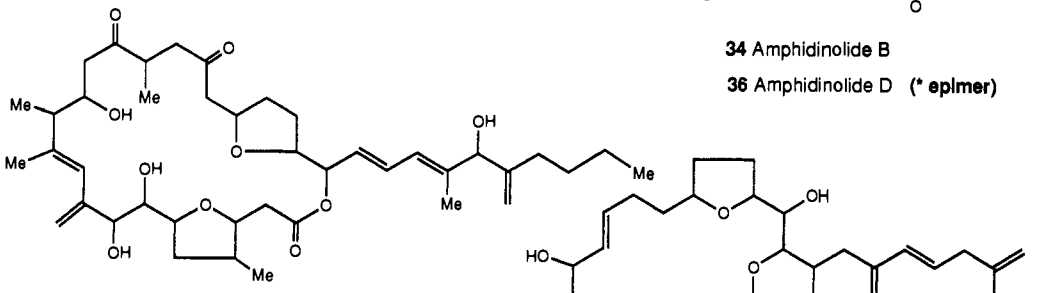


33 Amphidinolide A



34 Amphidinolide B

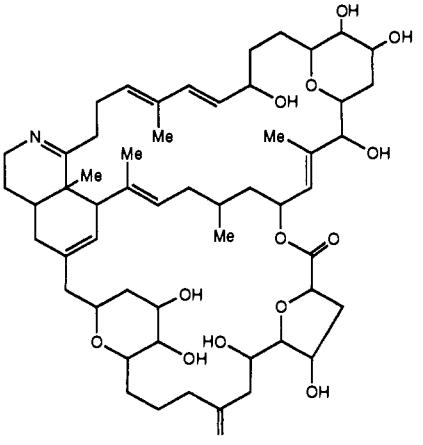
36 Amphidinolide D (* epimer)

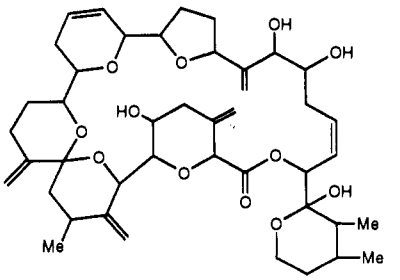


35 Amphidinolide C

37 Amphidinolide E

Chart VI





39 Goniodomin A

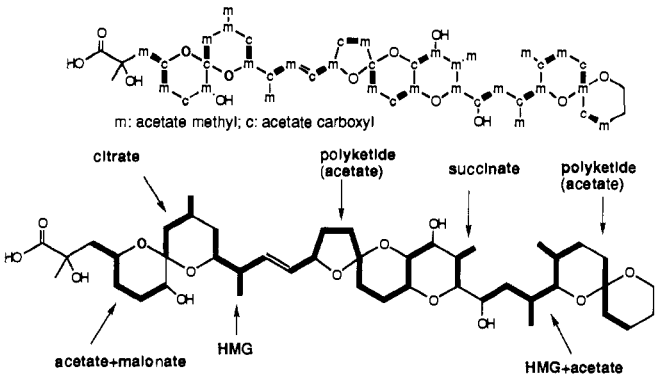


Figure 7. Possible building blocks of okadaic acid as judged from acetate feeding in *Prorocentrum lima*.[57]

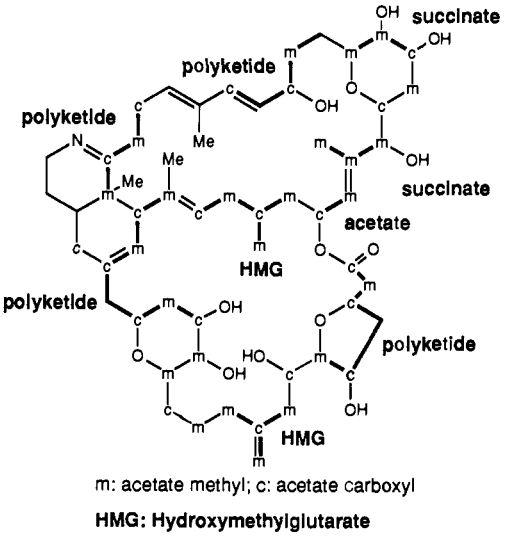


Figure 8. Acetate incorporation patterns in prorocentrolide (**38**) and possible building blocks.[57]

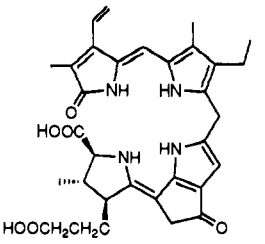
Although those compounds structurally resemble antibiotic metabolites produced by *Streptomyces* spp. and fungi, their biosynthetic origins seem to be quite different as in the case of brevetoxin type compounds. In fact, Yasumoto and co-workers fed ^{13}C-labeled acetate to the culture of *P. lima* and isolated okadaic acid (**32**) and prorocentrolide (**38**), which had labeling patterns unexplainable by the simple acetate condensation mechanism (Figures 7 and 8).[57] The finding is very similar to what was observed in the studies of brevetoxin biosynthesis and suggests the formation of the carbon chains from mixed building blocks including dicarboxylic acids.

It is highly intriguing that dinoflagellates synthesize polyketides in a different manner, as if they were organisms of different planets. Their peculiar biochemistry is probably due to their unique living environments, which have made them adopt metabolic pathways to utilize the most available compounds.

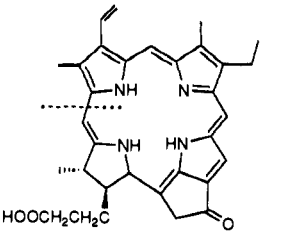
D. Compounds in Bioluminescence and Circadian Cycles

As indicated earlier, many dinoflagellates are bioluminescent, and the largest group of contributors to luminescence seen in the oceans. Although numerous studies have been done on the biological aspects of this fascinating phenomenon, the chemical basis of the dinoflagellate bioluminescent system was not known until very recently.

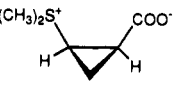
Chart VII



43



44



45

A group at Harvard University has recently determined the chromophore, which is responsible for the bioluminescence of the well-known luminescent organism, *Pyrocystis lunula*.[56] The compound was a bile pigment (**43**), which is closely related to pyropheophorbide *a* (**44**, Chart VII). The compound is very likely a degradation product of chlorophyll *a*, although we can not exclude the possibility that it is derived from a precursor of the chlorophyll biosynthesis.

An important, well-studied physiological property of dinoflagellates is their precise circadian rhythms, which control bioluminescence, photosynthesis, and cell division.[59] A chemical determinant of the periodicity in the dinoflagellate, *Gonyaulax polyedra* has been isolated by the same group.[60] The compound, gonyaulin (**45**), is a dimethylsulfonium derivative of cyclopropane carboxylic acid, which is probably biosynthesized from methionine.

IV. Diatom Metabolites

Diatoms comprise the largest population of microalgae in the oceans, but very few secondary metabolites of diatoms have been reported in the literature. Most of the reported organic components of diatoms are such common products as pigments, lipids, and carbohydrates. It is very likely that some earlier researchers simply decided, after a few futile attempts, that the chemistry of diatoms is barren and uninteresting. However, some recent discoveries may change this view completely.

A. Excitotoxic Amino Acid, Domoic Acid

Domoic acid (**46**), the culprit of the shellfish poisoning called amnesic shellfish poisoning (ASP), was traced to the common diatom, *Nitzschia pungens*.[61-63] The compound, which was also reported in *Amphora coffeiformis*[64] and *Pseudonitzschia australis*,[65] is an excitatory glutamate agonist and said to cause damage to important glutamate receptors in the brain. The production of domoic acid by diatoms is extremely interesting, because the compound was first discovered as an anthelmintic principle in the macro red algae, *Chondria armata*.[66] A closely related compound, kainic acid (**47**), is also found in the red algae, *Digenea*

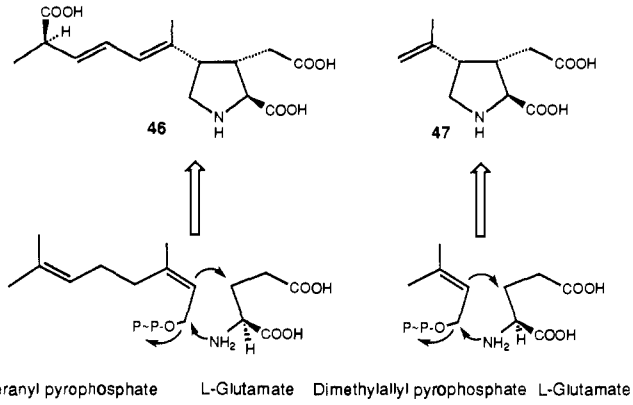


Figure 9. Proposed biosynthetic pathway of domoic acid (**46**) and kainic acid (**47**).

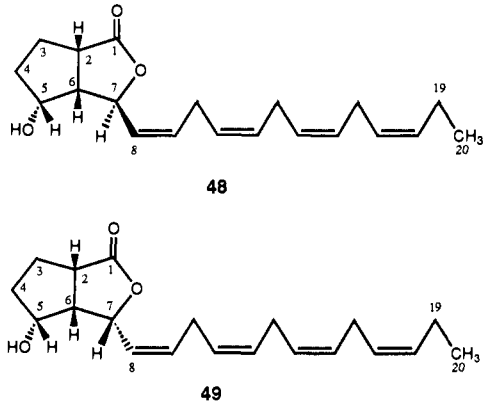
simplex.[67] The contribution of certain diatoms to the secondary metabolite production in macroalgae is only speculative, although it is known that the surface of many macroalgae is covered with benthic diatoms. On the other hand, there is a report that a dwarf strain of the red algae, *Palmaria palmata* produces kainic acid in an unialgal culture,[68] which may suggest the independent production of these compounds by the macroalgae.

The biosynthesis of domoic acid and kainic acid can be easily imagined to be formed by the condensation of glutamate and one or two prenyl groups. In fact, the stereochemistry of C-2 in domoic acid is identical with that of L-glutamate (Figure 9). Wright and his coworkers fed ^{13}C-labeled acetate and found that the labeling patterns found in domoic acid followed the biosynthesis of glutamate expected from acetate through the TCA cycle.[69]

B. Bacillariolides

Other interesting metabolites isolated from *N. pungens* are lactones, bacillariolide I (**48**) and II (**49**, Chart VIII).[70] They are a new type of cyclopentane eicosanoids, which are products by the ring closure at C-2 and C-6 of eicosapentaenoic acid (EPA) (Figure 10). It was suggested that the ring system is formed by perhydroxylation by 5-lipoxigenase, rearrangement to the epoxide, and the opening of the epoxide with anionic attack. The absolute configurations of the compounds were tentatively assigned according to the stereospecificity of the normal 5-lipoxigenase. However, it has been recently revealed by X-ray analysis of a derivative with an internal chiral marker that the absolute configurations are actually opposite to what were speculated on the basis of the biosynthetic pathway.[71] This raises an interesting question about the stereospecificity of 5-lipoxigenase in the diatom.

Chart VIII



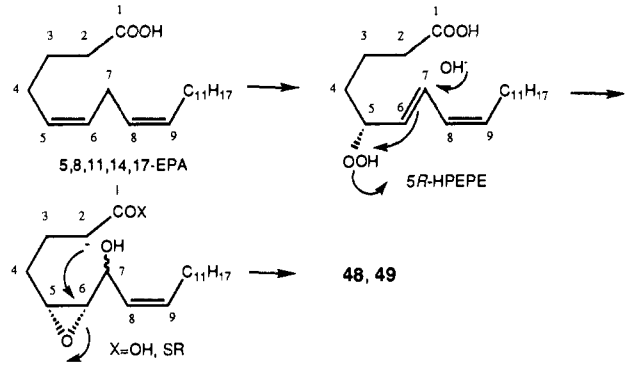
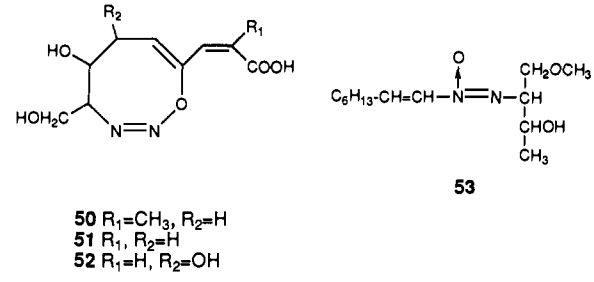


Figure 10. Proposed biosynthetic pathway of bacillariolide I (**48**) and II (**49**).

C. Asterionellins

The antibiotic activity shown by some diatoms was mostly attributed to rather common free fatty acid derivatives. However, a group of interesting compounds, asterionellin A (**50**), B (**51**), and C (**52**, Chart IX), with diazotate function have been recently found in the common diatom, *Asterionella* sp.[72] The unprecedented structures with cyclic vinyl diazotate group may need further studies for confirmation. The unusual diazotate group is isomeric to an azoxy group found in several antibiotics such as elaiomycin (**53**)[73] and probably has a similar biosynthetic origin.

Chart IX



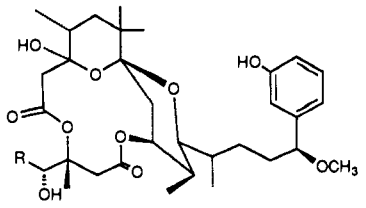
V. Blue-Green Algal Metabolites

A large number of interesting metabolites have been isolated from blue-green algae or cyanobacteria. Their chemical diversity is only comparable to the metabolites of Actinomycetes. However, most of the investigated algae are from freshwater or terrestrial origins, and only a limited number of marine blue-greens have been searched for secondary metabolites. Nevertheless, there are a number of hints that blue-greens are important players in the production of interesting compounds widely found in marine environments.

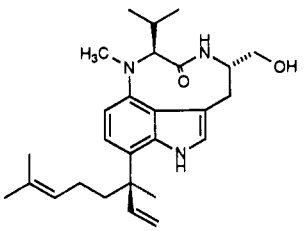
A. *Lyngbya* Toxins

Moore and his co-workers demonstrated that aplysiatoxin found in the sea slug *Aplysia* in Hawaiian water

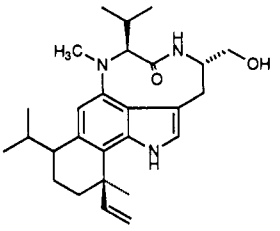
Chart X



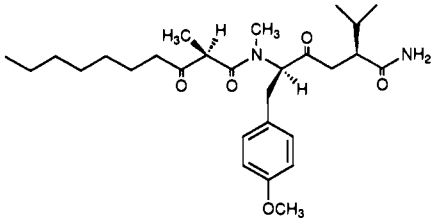
54 R=CH_3: debromoaplysiatoxin
55 R: oscillatoxin



56 Lyngbyatoxin A



57 Teleocidin B

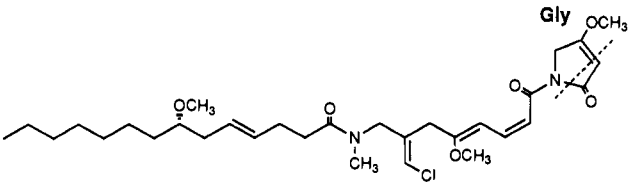


58 Majusculamide A

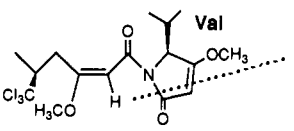
is actually derived from the filamentous blue-green, *Lyngbya majuscula*.[74] They isolated debromoaplysiatoxin (**54**, Chart X) from the wild population of the alga. The carbon skeleton of the aplysiatoxin resembles those of macrolide antibiotics,[75,76] but does not conform to the typical polyketide building blocks. For example, the *m*-cresol type aromatic ring does not fit the normal hydroxylation pattern of phenylpropanoid-or acetogenin-derived aromatic rings. Related compounds, oscillatoxins **55** found in a marine species of *Oscillatoria* sp. also have the same carbon skeleton.[77] *Lyngbya* produces other types of compounds. Lyngbyatoxins (**56**), which were isolated from *L. majuscula* in Hawaii,[78] are closely related to teleocidins (**57**), antibiotic alkaloids from *Streptomyces*.[79] The compounds are evidently biosynthesized from tryptophane, valine, and prenyl groups.

Signatures which suggest the participation of blue-greens in the formation of various metabolites are seen in the structures of many marine compounds. For example, Moore's group reported unique fatty acid amide derivatives in *L. majuscula* from deep waters. The compounds, represented by majusculamide A (**58**),[80] have a peptidal portion made of valine and tyrosine, whereas malyngamides, represented by malyngamide A (**59**, Chart XI),[81] from a shallow-water variety, have a characteristic polyketide-like chain and 4-methoxy-Δ^3-pyrrolin-2-one. The latter seems to be a Claisen condensation product of acetate and glycine. Similar pyrrolidone ring systems derived from the condensation of amino acids and acetate are seen in many other marine metabolites. For example, dysidin

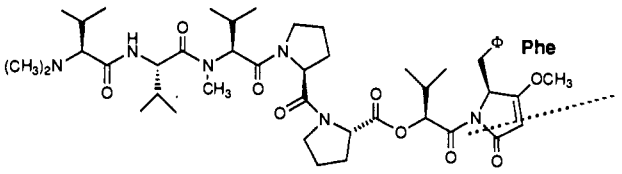
Chart XI



59 Malyngamide A



60 Dysidin



61 Dolastatin 15

(**60**), from the sponge, *Dysidia herbacea* has a ring system probably derived from valine and acetate.[82] It is very likely that the sponge metabolite has its origin in a blue-green alga, because *Dysidia* sponges are known to be associated with blue-greens. Similarly, an antitumor peptide, dolastatin 15 (**61**) from the sea hare, *Dolabella auricularia* contains a pyrrolidone moiety made of phenylalanine and acetate.[83]

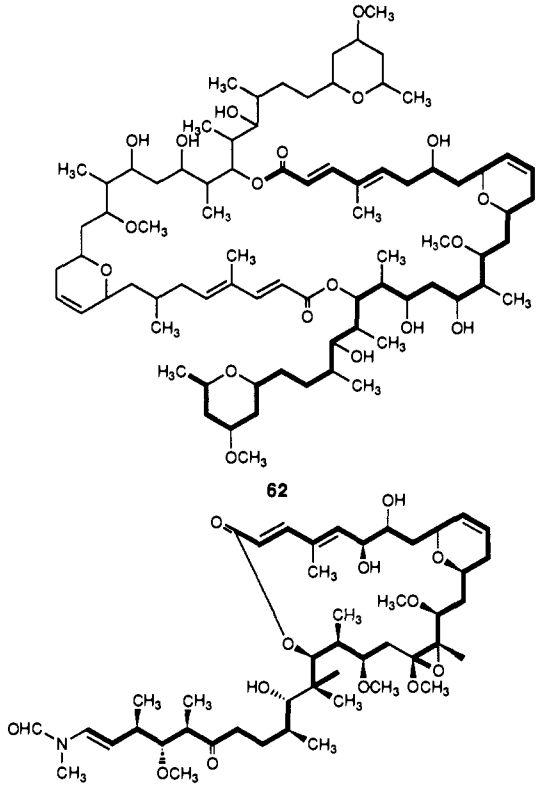
The involvement of amino acids in the biosynthesis of polyketide-type compounds are uncommon in terrestrial organisms, and it also suggests the uniqueness of the biosynthesis of algal metabolites. It is possible that some of the blue-green polyketides are biosynthesized in manners very different from its terrestrial counterparts, as in the case of polyether compounds in dinoflagellates. The geographical variation of chemical components within the same species as demonstrated by *Lyngbya* metabolites and the presence of different chemical strains are analogous to those of the Actinomycetes. Also, the occurrence of common metabolites such as lyngbyatoxins present an interesting question regarding the possible relationship between the blue-greens and Actinomycetes organisms.

B. Tolytoxin

Structural features of some macrolides found in marine invertebrates also suggests that the compounds have their origins in blue-green algae. For example, the bislactone, swinholide A (**62**),[84,85] and their related compounds isolated from *Theonella* sponges have the same skeleton as tolytoxin (**63**) or scytophycin found in terrestrial and littoral blue-green algae (Figure 11).[86]

C. Cyclic Peptides

A great number of cyclic peptides have been found in freshwater blue-greens, but only a few marine species have been investigated. From the brackish water species, *Nodularia spumigena*, which caused problems in Baltic Sea and New Zealand, cyclic pentapeptide,



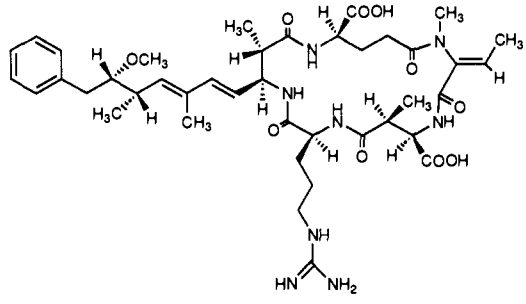
62

63

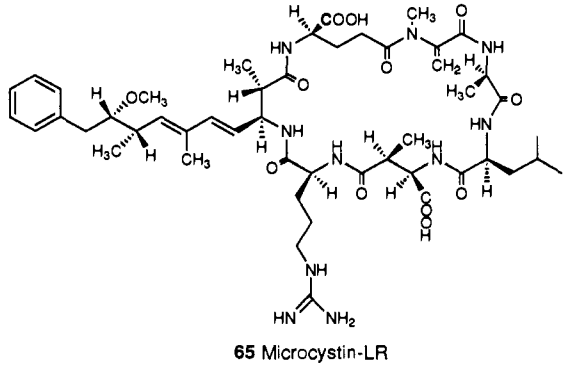
Figure 11. Skeletal resemblance demonstrated by a sponge constituent, swinholide (**62**) and a blue-green metabolite, tolytoxin (**63**).

nodularin (**64**, Chart XII) was isolated.[87] The compound is closely related to microcystins (**65**), potent hepatotoxin and protein phosphatase 2 and 2A inhibitors from the fresh-water blue-green algae, *Microcystis aeruginosa*.[88] The structures of both microcystins and nodularin are characterized by the presence of two unusual amino acids, 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid (Adda) (**66**) and 3-methylaspartic acid (Masp) (**67**).

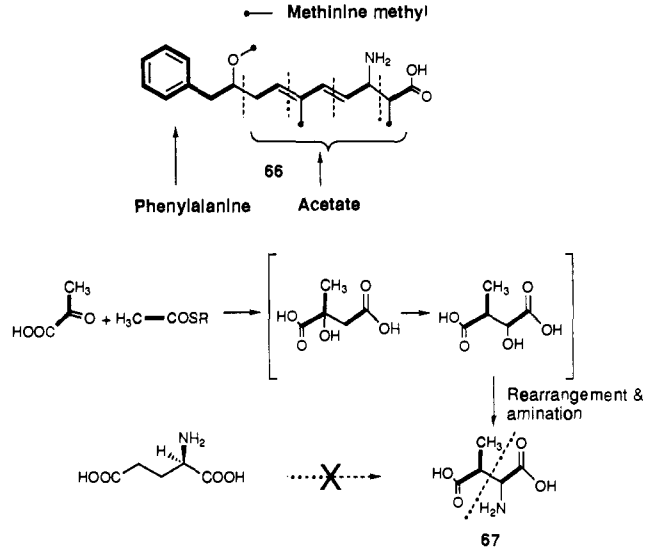
Chart XII



64 Nodularin



65 Microcystin-LR



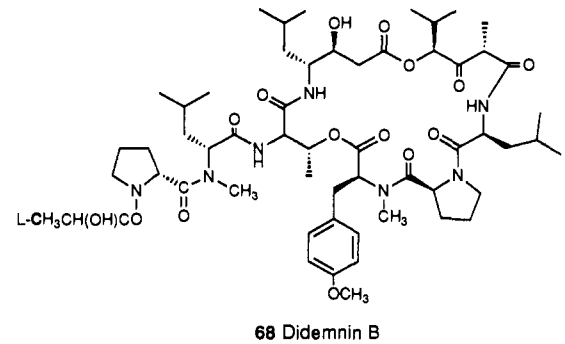
67

Figure 12. The biosynthetic building blocks of the β-amino acid, Adda (**66**) and methylaspartic acid (**67**) found in nodularin (**64**) and microcystins (**65**).[89]

Moore and his co-workers studied the biosynthesis of the moieties[89] and concluded that the former is a mixed polyketide made of phenylalanine (as phenylacetate), acetate, and methyl groups from methionine. A surprising finding in their work is that the algal (2*R*,3*S*)-3-methylaspartate (**67**) is formed by the condensation of pyruvate and acetate followed by rearrangement and amination (Figure 12). This is drastically different from the known pathway, which is the rearrangement of glutamate by methylaspartate mutase. This is another demonstration that the microalgae sometimes take completely different and unexpected paths to synthesize the same structures.

There is widespread speculation that many of the cyclic peptides found in tunicates and other marine invertebrates have their origin in symbiotic blue-greens or closely related organisms, prochlorons.[90] For example, it is speculated that the symbiotic prochloron in the tunicate, *Didemnum* sp. is totally or partially responsible for the production of didemnins represented by the strongly antiviral and antitumor agent, didemnin B (**68**, Chart XIII).[91] Many *Didemnum* species, which are symbiotic with prochlorons, also produce several

Chart XIII



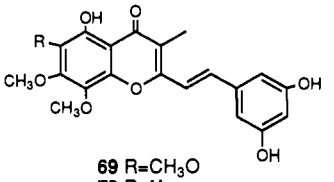
68 Didemnin B

other cyclic peptides. However, efforts to cultivate the symbiotic prochloron and to test this hypothesis have been so far unsuccessful.

VI. Other Microalgal Metabolites

Very little work has been done with such marine algae as Cryptophyceae. The Puerto Rican marine cryptophyte, *Chrysophaeum taylori*, produces an interesting styrylchromone derivatives, hormothamnione (**69**) and its desmethoxy derivative **70** (Chart XIV). The strongly

Chart XIV



69 R=CH_3O
70 R=H

cytotoxic compounds have a typical acetogenin-derived styryl group, but the oxygenation pattern of the chromone moiety does not necessarily conform to that of acetogenins. Until now, no analogous compounds have been isolated from other sources.

VII. Conclusion

With tens of thousands unexplored species and an infinite number of possible chemovars, marine microalgae seem to be a very promising source of useful compounds. The uniqueness of chemistry and metabolism of marine microalgae has been demonstrated with several types of metabolites. Also, there is strong evidence that many interesting compounds found in marine environments have their origins in microalgae. However, the chemistry of marine microalgae is still at its dawn. The slow progress is mainly due to difficulties in culturing the organisms. With a few exceptions, it is not feasible to do chemical work with material from the natural population of marine microalgae. At present, many important organisms remain unculturable despite enormous efforts. The dinoflagellates, *Dinophysis* spp. and ascidian prochlorons are among them. To make the matter worse, even if the organisms are successfully cultured, they often fail to produce desired compounds in culture. Thus, the progress of the chemistry of microalgae has to come with the progress of culture techniques and better understanding of their metabolism. Another big issue in this area is global concerns on the increasing incidents of toxic microalgal blooms (red tides), which pose serious threats to public health and fisheries. Although we have seen immense progress in recent years, many questions remain unanswered with regard to the trigger mechanism for the algal blooms and the toxigenicity. Again, concerted efforts are needed to solve this very complex problem.

VIII. References

(1) Yasumoto, T.; Murata, M. *Chem. Rev.* **1993**, this issue.
(2) Kobayashi, J. *Chem. Rev.* **1993**, this issue.
(3) Gerwick, W. H. *Chem. Rev.* **1993**, this issue.
(4) Faulkner, D. J. *Nat. Prod. Rep.* **1984**, *1*, 251, 551; **1986**, *3*, 1; **1987**, *4*, 539; **1988**, *5*, 613; **1990**, *7*, 269; **1991**, *8*, 97; **1992**, *9*, 323.
(5) For example, see: Sieburth, J. McN. *Sea Microbes*: Oxford University Press: New York, 1979.
(6) Corliss, J. O. In *Handbook of Protoctista*; Margulis, L., Corliss, J. O., Melkonian, M., Chapman, D. J., McKhann, H. I., Eds.; Jones and Bartlett: Boston, 1989; pp xi–xxx.
(7) *The Biology of Dinoflagellates*; Taylor, F. J. R., Ed.; Blackwell: Oxford, 1987.
(8) Dodge, J. D. *Prog. Protozool.* **1965**, *2*, 264.
(9) Sommer, H.; Meyer, K. F. *Arch. Pathol.* **1937**, *24*, 560.
(10) Shimizu, Y. In *Marine Natural Products, Chemical and Biological Perspectives*; Scheuer, P. J., Ed.; Academic Press: New York, 1978; pp 1–42.
(11) Shimizu, Y. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Grisebach, H., Kirby, G. W., Eds.; Springer: New York, 1984; pp 235–264.
(12) Hall, S.; Strichartz, G.; Moczydlowski, E.; Ravindran, A.; Reichardt, P. B. In *Marine Toxins*; Hall, S., Strichartz, G., Eds.; ACS Symposium Series 418; American Chemical Society: Washington, DC, 1987; pp 29–65.
(13) Harada, T.; Oshima, Y.; Kamiya, Y.; Yasumoto, T. *Jpn. Soc. Sci. Fish.* **1982**, *48*, 821.
(14) Jackim, E.; Gentile, J. *Science* **1968**, *162*, 915.
(15) Alam, M.; Shimizu, Y.; Ikawa, M.; Sasner, J. J. *J. Environ. Sci. Health* **1978**, *A13*, 493.
(16) Ikawa, M.; Wegener, K.; Faxall, T. L.; Sasner, J. J., Jr. *Toxicon* **1982**, *20*, 747.
(17) Oshima, Y.; Sugino, K.; Itakura, H.; Hirota, M.; Yasumoto, T. In *Toxic Marine Phytoplankton*; Graneli, E., Sundstrom, S., Edler, L., Anderson, E. M., Eds.; Elsevier: New York, 1989; pp 391–396. Oshima, Y.; Itakura, H.; Lee, K.; Yasumoto, Y.; Blackburn, S.; Hallegraeff, G. In *Toxic Phytoplankton Blooms in the Sea*; Smayda, T. J., Shimizu, Y., Eds.; Elsevier: Amsterdam, 1993; pp 907–912.
(18) Shimizu, Y.; Kobayashi, M.; Genenah, A.; Ichihara, N. In *Seafood Toxins*; Ragelis, E. P., Ed.; ACS Symposium Series 262; American Chemical Society: Washington, DC, 1984; pp 151–160.
(19) Shimizu, Y.; Norte, M.; Hori, A.; Genenah, A.; Kobayashi, M. *J. Am. Chem. Soc.* **1984**, *106*, 6433.
(20) Gupta, S.; Norte, M.; Shimizu, Y. *J. Chem. Soc., Chem. Commun.* **1989**, 1421.
(21) Shimizu, Y.; Gupta, S.; Chou, H. N. In *Marine Toxins*; Hall, S., Strichartz, G., Eds.; ACS Symposium Series 418; American Chemical Society: Washington, DC, 1987; pp 21–28.
(22) Shimizu, Y.; Gupta, S.; Prasad, A. V. K. In *Toxic Marine Phytoplankton*; Graneli, E., Sundstrom, S., Edler, L., Anderson, E. M., Eds.; Elsevier: New York, 1989; pp 62–73.
(23) Kobayashi, M.; Shimizu, Y. *J. Chem. Soc., Chem. Commun.* **1981**, 827.
(24) Sullivan, J. J.; Iwaoka, W. T.; Liston, J. *Biochem. Biophys. Res. Commun.* **1983**, *114*, 465.
(25) Shimizu, Y.; Yoshioka, M. *Science* **1980**, *212*, 547.
(26) Kotaki, Y.; Oshima, Y.; Yasumoto, T. *Bull. Jpn. Soc. Sci. Fish.* **1985**, *51*, 1009.
(27) Shimizu, Y.; Alam, M.; Fallon, W. E. In *Food-Drugs from the Sea Proceedings*; Webber, J. H., Ruggieri, G. D., Eds.; Marine Technology Society: Washington, DC, 1976; pp 238–251.
(28) Baden, D. G.; Mende, T. J.; Block, R. E. In *Toxic Dinoflagellate Blooms*; Taylor, D. L., Seliger, H. H., Eds.; Elsevier/North-Holland: New York, 1979; pp 307.
(29) Risk, M.; Lin, Y. Y.; MacFarlane, R. D.; Sadagopa-Ramanujam, V. M.; Smith, L. L.; Trieff, N. In *Toxic Dinoflagellate Blooms*; Taylor, D. L., Seliger, H. H., Eds.; Elsevier/North-Holland: New York, 1979; pp 335–344.
(30) Lin, Y. Y.; Risk, M.; Ray, S. M.; Van Engen, E.; Clardy, J.; Golik, J.; James, J. C.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, *103*, 6773.
(31) Shimizu, Y.; Chou, H. N.; Bando, J.; Van Duyne, T.; Clardy, J. *J. Am. Chem. Soc.* **1986**, *108*, 514.
(32) Prasad, A. V. K.; Shimizu, Y. *J. Am. Chem. Soc.* **1989**, *111*, 6476.
(33) Shimizu, Y. In *Natural Toxins*; Harris, J. B., Ed.; Clarendon Press: Oxford, 1986; pp 115–125.
(34) Nakanishi, K. *Toxicon* **1985**, *23*, 473.
(35) Lee, M. S.; Repeta, D. S.; Nakanishi, K.; Zagorski, M. G. *J. Am. Chem. Soc.* **1986**, *108*, 7855.
(36) Chou, H. N.; Shimizu, Y. *J. Am. Chem. Soc.* **1987**, *109*, 2184.
(37) Shimizu, Y.; Wrensford, G. In *Toxic Phytoplankton Blooms in the Sea*; Smayda, T. J., Shimizu, Y., Eds.; Elsevier: Amsterdam, 1993; pp 907–912.
(38) Lee, M. S.; Quin, G-W.; Nakanishi, K.; Zagorski, M. G. *J. Am. Chem. Soc.* **1989**, *111*, 6234.
(39) Tachibana, K. Ph.D. Thesis, University of Hawaii, 1980.
(40) Murata, M.; Legrand, A. M.; Ishibashi, Y.; Fukui, M.; Yasumoto, T. *J. Am. Chem. Soc.* **1989**, *111*, 8929; **1990**, *112*, 4380.
(41) Nagai, H.; Torigoe, K.; Satake, M.; Murata, M.; Yasumoto, T.; Iwashita, T. *J. Am. Chem. Soc.* **1992**, *114*, 1102.
(42) Murata, M.; Iwashita, T.; Yokoyama, A.; Sasaki, M.; Yasumoto, T. *J. Am. Chem. Soc.* **1992**, *114*, 6594.
(43) Bialojan, C.; Takai, A. *Biochem. J.* **1988**, *256*, 283.
(44) Haystead, T. A. J.; Sim, A. T. R.; Carling, D.; Honnor, R. C.; Tsukitani, Y.; Cohen, P.; Hardie, D. G. *Nature* **1989**, *337*, 78.

(45) Kobayashi, J.; Ishibashi, M.; Nakamura, H.; Ohizumi, Y.; Yamasu, T.; Sasaki, T.; Hirata, Y. *Tetrahedron Lett.* **1986**, *27*, 5755.
(46) Ishibashi, M.; Ohizumi, Y.; Hamashima, M.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Kobayashi, J. *J. Chem. Soc., Chem. Commun.* **1987**, 1127.
(47) Kobayashi, J.; Ishibashi, M.; Wälchli, M. R.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Ohizumi, Y. *J. Am. Chem. Soc.* **1988**, *110*, 490.
(48) Kobayashi, J.; Ishibashi, M.; Murayama, T.; Takamatsu, M.; Iwamura, M.; Ohizumi, Y.; Sasaki, T. *J. Org. Chem.* **1990**, *55*, 3421.
(49) Torigoe, K.; Murata, M.; Yasumoto, T. *J. Am. Chem. Soc.* **1988**, *110*, 7876.
(50) Murakami, M.; Makabe, K.; Yamaguchi, K.; Konosu, S.; Wälchli, M. R. *Tetrahedron Lett.* **1988**, *29*, 1149.
(51) Tachibana, K.; Scheuer, P.; Tsukitani, Y.; Kikuchi, H.; Van Engen, D.; Clardy, J.; Gopichand, Y.; Schmitz, F. J. *J. Am. Chem. Soc.* **1981**, *103*, 2469.
(52) Murakami, Y.; Oshima, Y.; Yasumoto, T. *Bull. Jpn. Soc. Sci. Fish.* **1982**, *48*, 69.
(53) Dickey, R. W.; Borzin, S. C.; Faulkner, D. J.; Bencsath, F. A.; Andrzejewski, D. *Toxicon* **1990**, *28*, 371.
(54) Yasumoto, T.; Oshima, Y.; Sugawara, W.; Fukuyo, Y.; Oguri, H.; Igarashi, T.; Fujita, N. *Bull. Jpn. Soc. Sci. Fish.* **1980**, *46*, 1405.
(55) Schmitz, F. J.; Prasad, R. S.; Gopichand, Y.; Jossain, M. B.; van der Helm, D. *J. Am. Chem. Soc.* **1981**, *103*, 2467.
(56) Yasumoto, T.; Murata, M.; Oshima, Y.; Matsumoto, G. K.; Clardy, J. In *Seafood Toxins*; Ragelis, E. P., Ed.; ACS Symposium Series 262; American Chemical Society: Washington, DC, 1984; pp 207–214.
(57) Yasumoto, T.; Torigoe, K. *Abstract of Papers*; U.S.-Japan Seminar, Bioorganic Marine Chemistry, Honolulu, HI, 1990, *J. Nat. Prod.* **1991**, *54*, 1487.
(58) Nakamura, H.; Kishi, Y.; Shimomura, O.; Morse, D.; Hastings, J. W. *J. Am. Chem. Soc.* **1989**, *111*, 7607.
(59) Sweeney, B. M. In *The Biology of Dinoflagellates*; Taylor, F. J. R., Ed.; Blackwell: Oxford, 1987; pp 269–281.
(60) Roenneberg, T.; Nakamura, H.; Cranmer, L. D., III; Ryan, K.; Kishi, Y.; Hastings, J. W. *Experientia* **1991**, *47*, 103.
(61) Atlantic Research Laboratory Technical Report 56, NRCC 29083; 57, NRCC 29086; Atlantic Research Laboratory, NRC: Halifax, Canada, 1988.
(62) Subba Rao, D. V.; Quilliam, M. A.; Pocklington, R. *Can. J. Fish. Aquat. Sci.* **1988**, *45*, 2076.
(63) Maranda, L.; Wang, R.; Masuda, K.; Shimizu, Y. In *Toxic Marine Phytoplankton*; Graneli, E., Sundstrom, S., Edler, L., Anderson, E. M., Eds.; Elsevier: New York, 1989; pp 300–304.
(64) Shimizu, Y.; Gupta, S.; Masuda, K.; Maranda, L.; Walker, C. R.; Wang, R. *Pure Appl. Chem.* **1989**, *61*, 513.
(65) Fritz, L.; Quilliam, M. A.; Wright, J. L. C.; Beale, A. M.; Work, T. M. *J. Phycol.* **1992**, *28*, 439.
(66) Daigo, K. *Yakugaku Zasshi* **1959**, *79*, 350; 353; 356.
(67) Murakami, S.; Takemoto, T.; Shimizu, S. *Yakugaku Zasshi* **1953**, *73*, 1026.
(68) Laycock, M. V.; Bird, C. J. *Abstracts of Papers*, 13th International Seaweed Symposium, Vancouver, Canada, 1989; A-87. Laycock, M. V.; de Freitas, A. S. W.; Wright, J. L. C. *J. Appl. Phycol.* **1989**, *1*, 113.
(69) Ramsey, U.; Douglas, D.; Douglas, S.; Walter, J. A.; Wright, J. L. C. *Abstracts of Papers*, 2nd International Marine Biotechnology Conference, Baltimore, MD, 1991; p 70; *J. Chem. Soc., Chem. Commun.* **1992**, 714.
(70) Wang, R.; Shimizu, Y. *J. Chem. Soc., Chem. Commun.* **1990**, 413.
(71) Wang, R.; Shimizu, Y.; Rios Steiner, J. R.; Clardy, *J. Chem. Soc., Chem. Commun.* **1993**, 379.
(72) Wang, R. Ph.D. Thesis, University of Rhode Island, 1992.
(73) Stevens, C. L.; Gillis, B. T.; French, J. C.; Haskell, T. H. *J. Am. Chem. Soc.* **1958**, *80*, 6088.
(74) Mynderse, J. S.; Moore, R. E.; Kashiwagi, M.; Norton, T. R. *Science* **1977**, *196*, 538.
(75) Moore, R. E.; Blackman, A. J.; Ceuk, C. E.; Mynderse, J. S.; Matsumoto, G. K.; Clardy, J.; Woodard, R. W.; Craig, J. C. *J. Org. Chem.* **1984**, *49*, 2484.
(76) Kato, Y.; Scheuer, P. J. *Pure Appl. Chem.* **1975**, *41*, 1.
(77) Mynderse, J. S.; Moore, R. E. *J. Org. Chem.* **1978**, *43*, 2301.
(78) Cardellina, J. H.; Marner, F.-J.; Moore, R. E. *Science* **1979**, *204*, 193.
(79) Sakai, S.; Hitosuyanagi, Y.; Aimi, N.; Fujiki, H.; Suganuma, M.; Sugiura, T.; Endo, Y.; Shudo, K. *Tetrahedron Lett.* **1986**, *27*, 5219.
(80) Marner, F.-J.; Moore, R. E.; Hirotsu, K.; Clardy, J. *J. Org. Chem.* **1977**, *42*, 2815.
(81) Cardellina, J. H., II; Marner, F.-J.; Moore, R. E. *J. Am. Chem. Soc.* **1979**, *101*, 240.
(82) Hofheinz, W.; Oberhänsli, W. E. *Helv. Chim. Acta* **1977**, *60*, 660.
(83) Pettit, G. R.; Kamano, Y.; Dufresne, C.; Cerny, R. L.; Herad, C. L.; Schmidt, J. M. *J. Org. Chem.* **1989**, *54*, 6005.
(84) Carmely, S.; Kashman, Y. *Tetrahedron Lett.* **1985**, *26*, 511.
(85) Kobayashi, M.; Tanaka, J.; Katori, T.; Matsuura, M.; Kitagawa, I. *Tetrahedron Lett.* **1989**, *30*, 2963.
(86) Carmely, S.; Moore, R. E.; Patterson, G. M. L. *J. Nat. Prod.* **1990**, *53*, 1533.
(87) Rinehart, K. L.; Harada, K.; Namikoshi, M.; Chen, C.; Harvis, C. A.; Munro, M. H. G.; Blunt, J. W.; Mulligan, P. E.; Beasley, V. R.; Dahlem, A. M.; Carmichael, W. W. *J. Am. Chem. Soc.* **1988**, *110*, 8557.
(88) Carmichael, W. W. In *Handbook of Natural Toxins*; Tu, A. T., Ed.; Marcel Dekker: New York, 1988; Vol. 3, pp 121–147.
(89) Moore, R. E.; Chen, J. L.; Moore, B. S.; Patterson, G. M. L.; Carmichael, W. W. *J. Am. Chem. Soc.* **1991**, *113*, 5083.
(90) *Prochloron*; Lewin, R. A., Cheng, L., Ed.; Chapman and Hall: New York, 1989.
(91) Rinehart, K. L.; Kishore, V.; Bible, K. C.; Sakai, R.; Sullins, D. W.; Li, K.-M. *J. Nat. Prod.* **1988**, *51*, 1.
(92) Gerwick, W. H.; Lopez, A.; Van Duyne, G. D.; Clardy, J.; Ortiz, W.; Baez, A. *Tetrahedron Lett.* **1986**, *27*, 1979.
(93) Gerwick, W. H. *J. Nat. Prod.* **1989**, *52*, 252.