Journal of Industrial Microbiology (1996) 17, 385–396

Compounds produced from potential tunicate-blue-green algal symbiosis: a review

HL Sings and KL Rinehart

Roger Adams Laboratory, University of Illinois at Urbana-Champaign, 600 S Mathews Ave, Urbana, IL 61801, USA

Tunicates of the family Didemnidae can be host to two genera of blue-green algae: *Synechocystis* and *Prochloron*. The presence of symbiotic algae raises questions as to the exact origin of the biologically important metabolites which have been isolated from tunicates in recent years. Is the compound produced by the tunicate, the alga, or through a combined effort of both organisms? Although this question cannot be fully addressed at the present time, there is evidence which supports the argument that the metabolic origin of certain ascidian metabolites resides in the alga, or is due to a collaborative effort of both organisms. The purpose of this review is to present compounds isolated from tunicates that possess a likely symbiotic relationship with either *Synechocystis* or *Prochloron*. Attention will also be given to the ecology of the organisms and the biological activities of metabolites isolated.

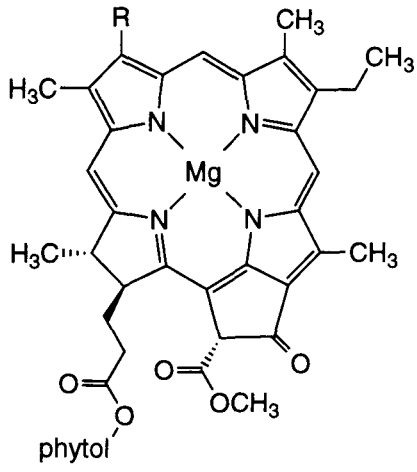
Keywords: cyanobacteria; ascidian; *Synechocystis*; *Prochloron*; marine natural product

Marine organisms of the class Ascidiacea, also referred to as tunicates or sea squirts, are hermaphroditic animals belonging to the phylum Chordata, subphylum Tunicata. They are called tunicates because the adult body is entirely embedded within a tunic, which forms the exoskeleton, and called sea squirts for their ability to expel water through siphons which connect the hollow adult body to the marine environment. Ascidian physiology and chemistry have been studied extensively, and are the subject of several excellent books and reviews [11,19,46,47]. However, it is their symbiotic association with prokaryotic microalgae that is the topic of this report. Most tunicates have a well-developed immune system [8] and do not possess endobionts; however, some species of tunicates can be host to blue-green alga, in either of the *Synechocystis* or *Prochloron*-genera [38]. This symbiosis is most often found within the family Didemnidae, the dominant ascidian family in shallow tropical habitats. The presence of algae is therefore often used as a diagnostic criterion in the taxonomy of the Didemnidae family. To date, all the algal species living within ascidian tissue examined are prokaryotic, with the exception of six species of ascidians from the cold waters of southern New Zealand, which may harbor eukaryotic and/or prokaryotic algae—*Asterocarpa humilis* (Styelidae), *Pyura cancellata*, *P. carnea*, *P. pulla*, *P. suteri* (Pyuridea), and *Aplidium thomsoni* (Polyclinidae), and two species of tunicates from Nantes, France—*Phallusia mammillata* and *Ascidia mentula* [38].

Blue-green algae, or cyanobacteria, are a group of prokaryotic photosynthetic microorganisms which, like plants, utilize H_2O as their photosynthetic electron donor [76]. This is in contrast to purple and green photosynthetic bacteria that use substrates such as H_2 and H_2S, a more primitive form of photosynthesis [76]. Thus, in evolutionary terms, blue-green algae represent a link between bacteria and green plants. Blue-green algal fossils date back to the Early Precambrian period, at which time they were probably the primary producers of organic matter and the first organisms to release molecular O_2 into the atmosphere [15]. *Synechocystis* can be found in all tropical oceans, the Gulf of Mexico, and among the islands of the Caribbean Sea [46]. Some of the known associations of *Synechocystis* with didemnids include *Didemnum candidum*, *D. digestum*, *Tridemnum solidum*, *T. miniatum*, *T. cyanophorum*, and *T. cerebriforme* [36,53]. Phylogenetically, *Prochloron* is a member of the blue-green algae radiation, but is distinguished from other cyanophytes by the absence of phycobilisomes, high molecular weight complexes composed of proteins and linear tetrapyrroles [76], and by the presence of chlorophyll b in addition to chlorophyll a (Figure 1) [40]. The first *Prochloron* to be identified, *P. didemni*, was originally thought to be an odd *Synechocystis*. Further investigation into its photosynthetic components led to a new division of algae termed Prochlorophyta, based on the generic name *Prochloron*. The validation of this species, and its eventual placement among the Cyanophyta, did not occur until 1993 [26]. Since the description of *Prochloron didemni*, two other Prochlorophytes have been discovered and named: *Prochlorothrix hollandica* and *Prochlorococcus marinus* [26]. *Prochloron* sp have been found on the surface, or in the common cloacal cavity of didemnids such as *Lissoclinum patella*, *L. bistratum*, *L. voeltzkowi*, *L. punctatum*, *T. cyclops*, *T. clinides*, *D. molle*, and *Diplosoma virens* [39,51].

In certain didemnid species, the symbiosis appears to be obligate for the tunicate. The algae may reside inside the tissues, within the tunic, or on the surface of the host [46]. It is therefore not surprising that the tunic is often transparent, allowing for photosynthesis to occur while at the same time providing a protective covering. An obligate symbiosis in didemnids is evident in the morphological adaptations exhibited by the larvae. The larvae contain special pouches in which they transmit algae to successive gen-

Correspondence: KL Rinehart, Roger Adams Laboratory, University of Illinois at Urbana-Champaign, 600 S Mathews Ave, Urbana, IL 61801, USA
Received 8 April 1996; accepted 30 August 1996



chlorophyll a R = $CH{=}CH_2$
chlorophyll b R = CHO

Figure 1 Chlorophylls a and b.

erations of didemnids, thereby ensuring perpetuation of the symbiosis [34,35]. Furthermore, several studies have demonstrated that the algae can furnish some organic materials to the host, and are capable of supporting a significant fraction of the ascidians' energy demands [21,29,52]. It was also noted by Lewin and Cheng that marine animals do not feed on symbiotic didemnids, presumably due to cytotoxic constitutents produced by the symbiosis.

Tunicates have proven to be a rich source of structurally intriguing, biologically important compounds. For example, the didemnins, from *Tridemnum solidum*, are a class of cyclic depsipeptides showing antitumor, antiviral, and potent immunosuppressive activity (Figure 2) [23,60,61,62,64,65]. Didemnin B was the first drug candidate from a marine species to enter Phase I and Phase II clinical trials. Like *Tridemnum solidum*, many ascidians, from which biologically active metabolites have been isolated, are in symbiosis with blue-green algae. The compounds are generally attributed to the animal; however, the presence of symbiotic algae raises questions as to the exact origin of these metabolites. Is the compound produced by the tunicate, the alga, or through a combined effort of both organisms? This question is difficult to address for several reasons: (1) a clear symbiotic relationship must be established; (2) examination of the chemical composition of the individual organisms is often hindered by the inability to completely separate the algae from the host; (3) attempts at culturing symbiotic algae, especially *Prochloron*, have been largely unsuccessful [39]; (4) when removed from its host, a cultured symbiont may not produce the same secondary metabolites; and (5) most authors, when reporting a new ascidian metabolite, fail to mention if the ascidian possesses an algal symbiont.

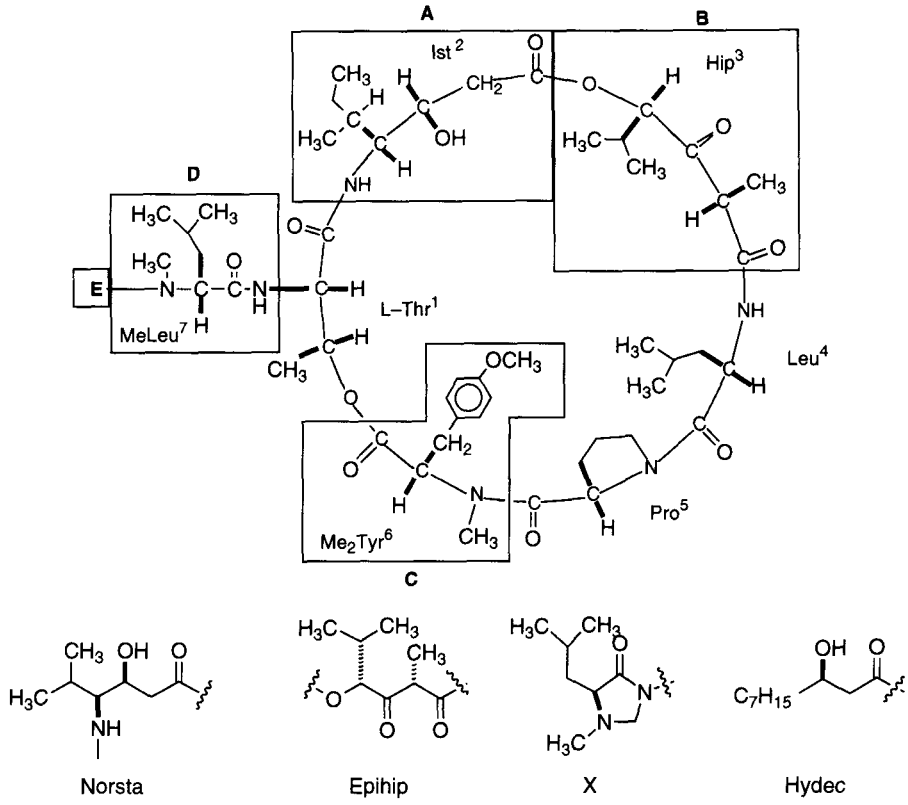
Although the question of metabolic origin cannot be fully addressed at the present time, there are some studies which support the concept that symbiotic microalgae may produce some of the molecules which have been isolated from marine ascidians. In the marine natural product literature, compounds are suggested to be of algal origin: if they (1) are found in isolated algal cells; (2) are found in other taxonomic groups known to harbor blue-green algae; (3) are present in free-living cyanobacteria; or (4) contain structural motifs which cannot be ascribed to the invertebrate.

These assumptions are not limited to tunicates which harbor blue-green algae. A recent report identified several metabolites from an unidentified Western Australian *Didemnum* which are related to the microbial antibiotic enterocin [30]. Although the authors were unable to isolate these metabolites from cultured bacteria, there was a direct correlation between the presence of large numbers of morphologically-distinct bacteria and, according to the authors, the isolation of 'what are most likely microbial metabolites.' In that case, as in most of the cases covered in the present review, there was evidence which supported algal origin, but this origin was not rigorously established. The purpose of the present review is to present compounds isolated from tunicates which possess a documented symbiotic relationship with either *Synechocystis* or *Prochloron*. For some of the metabolites presented, there is evidence which suggests algal origin, but often there is a lack of experimental data to support either side of the argument. Attention will also be given to the ecology of the organisms and the biological activities of isolated metabolites.

Tridemnum solidum

T. solidum is a colonial tunicate that is symbiotically associated with the blue-green alga *S. tridemni* [37]. The ascidian host appears to be physiologically dependent on its symbionts, as shown by morphological adaptations exhibited by the tunicate in response to ambient light levels [50]. *T. solidum* grown in full sunlight distributes *S. tridemni* more uniformly than shaded colonies, which concentrate their algae near the surface. Reduction of light beyond a certain level is lethal to the animals, but it is not known whether death results from insufficient photosynthetic production by *S. tridemni*, or toxicity due to the dying algae. Like other obligately symbiotic didemnids, *T. solidum* releases its larvae during daylight hours, a time optimal for the concomitant growth of the symbiont [49]. This is in contrast to ascidians that do not possess blue-green algae, which release their larvae at dawn or continuously over a day/night cycle. The algal-containing larvae of *T. solidum* induce vomiting in fish, resulting in a rapid learned aversion to this toxic food source [41].

Methanol-toluene (3 : 1) extracts of *T. solidum* collected during the *Alpha Helix* Caribbean Expedition 1978 showed promising anti-viral activity in shipboard assays [61]. Bioassay-guided separation of the crude extract led to the isolation of a new class of cyclic depsipeptides, didemnins A, B and C. Later, didemnins D, E, G, M, N, X and Y, nordidemnins A, B, and N, methylenedidemnin A, epididemnin A_1, and a ring-opened form of didemnin A, acyclodidemnin A, were isolated from the same source (Figure 2) [23,65]. Didemnin B was the first marine-derived natural product to enter clinical trials, and is currently finishing Phase II trials in the United States. Although numerous chemical, structural, and biological studies have been reported, the mechanism by which didemnin B induces its cytotoxic and immunosuppressive effect on the cell is still being developed [10,22,23,60,64,65,72]. In a recent report,

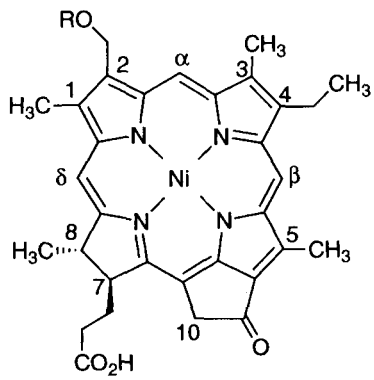


		A	B	C	D	E
Didemnins	A	Ist	Hip	Me_2Tyr^-	D-MeLeu	-H
	B	Ist	Hip	Me_2Tyr	D-MeLeu	-Pro-Lac
	C	Ist	Hip	Me_2Tyr	D-MeLeu	-Lac
	D	Ist	Hip	Me_2Tyr	D-MeLeu	-Pro-Lac-Gln-Gln-Gln-pGlu
	E	Ist	Hip	Me_2Tyr	D-MeLeu	-Pro-Lac-Gln-Gln-pGlu
	G	Ist	Hip	Me_2Tyr	D-MeLeu	-CHO
	M	Ist	Hip	Me_2Tyr	D-MeLeu	-Pro-Lac-Gln-pGlu
	N	Ist	Hip	Tyr	D-MeLeu	-Pro-Lac
	X	Ist	Hip	Me_2Tyr	D-MeLeu	-Pro-Lac-Gln-Gln-Gln-Hydec
	Y	Ist	Hip	Me_2Tyr	D-MeLeu	-Pro-Lac-Gln-Gln-Gln-Gln-Hydec
Dehydrodidemnin	B	Ist	Hip	Me_2Tyr	D-MeLeu	-Pro-Pyruv
Nordidemnins	A	Norsta	Hip	Me_2Tyr	D-MeLeu	-H-
	B	Norsta	Hip	Me_2Tyr	D-MeLeu	-Pro-Lac
	N	Norsta	Hip	Tyr	D-MeLeu	-Pro-Lac
Methylenedidemnin	A	Ist	Hip	Me_2Tyr	X	--
Epididemnin	A_1	Ist	epiHip	Me_2Tyr	D-MeLeu	-H
Acyclodidemnin	A	Ist	Hip	Me_2TyrOH	D-MeLeu	-H

Figure 2 The didemnins.

Grubb *et al* demonstrated that didemnin B exerts its cytotoxicity in HL-60 cells through the induction of apoptosis, or programmed cell death [22]. They report that didemnin B caused the most rapid and complete induction of apoptosis in HL-60 cells of any compound studied thus far, surpassing other chemotherapeutic agents such as cisplatin and taxol. In other studies, didemnin B cytotoxicity has been attributed to inhibition of protein synthesis [10,72]. Crews and coworkers have demonstrated that didemnin B binds to elongation factor (EF) 1α, a guanine nucleotide-binding protein that transports amino-acyl tRNAs to the ribosomal A site in a GTP-dependent manner. They report that the binding of didemnin B to EF-1α is GTP-dependent, but does not interfere with the GTPase activity of EF-1α. This observation suggests that EF-1α is the intracellular target responsible for didemnin B's ability to inhibit protein synthesis. In a more recent study, didemnin B inhibited protein biosynthesis in eukaryotic cell lysates by preventing EF-2 dependent translocation. Didemnin B is believed to prevent translocation by stabilizing aminoacyl-tRNA bound to the ribosomal A-site, similar to the antibiotic kirromycin. It is apparent didemnin B cytotoxicity may arise from more than one mechanism.

Didemnin B has also been isolated from a related

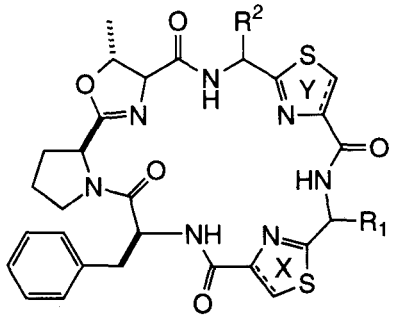


acyl-tunichlorins

R	
$C_{14}H_{25}O$	= 9-tetradecenoate
$C_{15}H_{27}O$	= 9-pentadecenoate
$C_{16}H_{29}O$	= 9-hexadecenoate
$C_{17}H_{31}O$	= 9-heptadecenoate
$C_{18}H_{33}O$	= 9-octadecenoate
$C_{18}H_{33}O$	=11-octadecenoate
$C_{19}H_{35}O$	=10-nonadecenoate
$C_{20}H_{37}O$	=13-eicosenoate

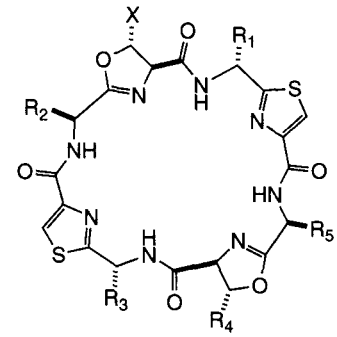
R	
$C_{14}H_{27}O$	= tetradecanoate
$C_{15}H_{29}O$	= pentadecanoate
$C_{16}H_{31}O$	= hexadecanoate
$C_{17}H_{33}O$	= heptadecanoate
$C_{18}H_{35}O$	= octadecanoate
$C_{19}H_{37}O$	= nonadecanoate
$C_{20}H_{39}O$	= eicosanoate
$C_{21}H_{41}O$	= heneicosanoate
$C_{22}H_{43}O$	= docosanoate
$C_{23}H_{45}O$	= tricosanoate
$C_{16}H_{27}O$	= 9, 12-hexadecadienoate
$C_{18}H_{31}O$	= 9, 12-octadecadienoate
$C_{19}H_{33}O$	= 9, 14-nonadecadienoate
$C_{20}H_{35}O$	= 10, 12-eicosadienoate
$C_{18}H_{29}O$	= 9, 12, 15-octadecatrienoate
$C_{20}H_{33}O$	= 7, 10, 13-eicosatrienoate
$C_{20}H_{29}O$	= 5, 8, 11, 14, 17-eicosapentaenoate
$C_{20}H_{31}O$	= 5, 8, 11, 14-eicosatetraenoate
$C_{22}H_{31}O$	= 4, 7, 10, 13, 16, 19-docosahexaenoate
$C_{22}H_{33}O$	= 7, 10, 13, 16, 19-docosapentaenoate

Figure 3 The acyl-tunichlorins.



	X	Y	R_1	R_2
ulicyclamide	thiazoline	thiazole	D-Val	L-Ile
lissoclinamide 1	thiazoline	thiazole	D-Ile	L-Val
lissoclinamide 2	thiazoline	thiazoline	D-Ala	D-Ile
lissoclinamide 3	thiazoline	thiazoline	L-Ala	D-Ile
lissoclinamide 4	thiazole	thiazoline	L-Phe	D-Val
lissoclinamide 5	thiazole	thiazole	L-Phe	D-Val
lissoclinamide 6	thiazole	thiazoline	D-Phe	D-Val
lissoclinamide 7	thiazoline	thiazoline	D-Phe	Val
lissoclinamide 8	thiazole	thiazoline	D-Phe	L-Val

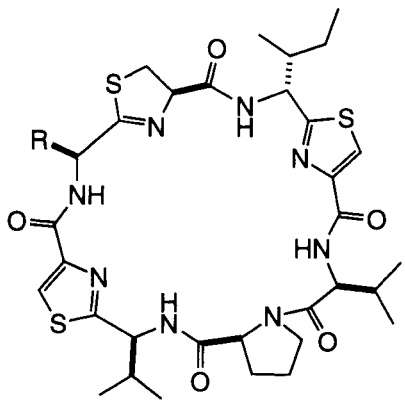
Figure 4 The lissoclinamides. For amino acid derived metabolites, R represents the side chain found in the parent amino acid listed.



name	X	R_1	R_2	R_3	R_4	R_5
ulithiacyclamide	Me	D-Leu	L-1/2 Cys_2	D-Leu	Me	L-1/2 Cys_2
ulithiacyclamide B	Me	D-Phe	L-1/2 Cys_2	D-Leu	Me	L-1/2 Cys_2
patellamide A	H	D-Val	L-Ile	D-Val	Me	L-Ile
patellamide B	Me	D-Ala	L-Leu	D-Phe	Me	L-Ile
patellamide C	Me	D-Ala	L-Val	D-Phe	Me	L-Ile
patellamide D	Me	D-Ala	L-Ile	D-Phe	Me	L-Ile
patellamide E	Me	D-Val	L-Val	D-Phe	Me	L-Ile
patellamide F	Me	D-Val	L-Val	D-Phe	H	D-Val
ascidiacyclamide	Me	D-Val	L-Ile	D-Val	Me	L-Ile

Figure 5 The patellamides. For amino acid derived metabolites, R represents the side chain found in the parent amino acid listed.

ascidian *Tridemnum cyanophorum*, which is also found in symbiotic association with *Synechocystis trididemni* [6]. In addition to didemnin B, *T. cyanophorum* has provided didemnin M (referred to by those authors as didemnin H), and a new addition to the family, [D-Pro^5]didemnin B [1]. Additionally, a Mediterranean tunicate, *Aplidium albicans*, has afforded dehydrodidemnin B [59,67]. Dehydrodidemnin B is three to five times as active *in vitro* as didemnin B and is as effective as didemnin B in treating leukemia (T/C = 210), melanoma (T/C = 218) and Lewis lung carcinoma (T/C = 0.0) in mice, all @ 160 $\mu g\ kg^{-1}$ $injection^{-1}$. It is expected to enter human clinical trials within a year.

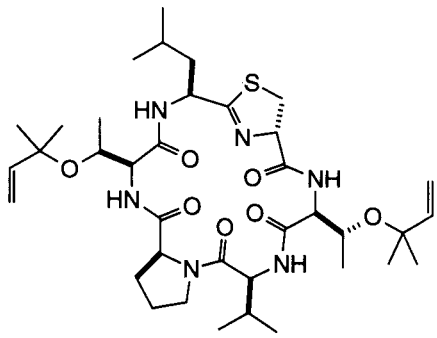


name	R
tawicyclamide A	L-Phe
tawicyclamide B	L-Leu

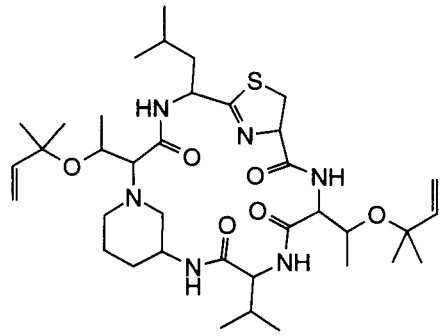
Figure 6 Tawicyclamides A and B. For amino acid derived metabolites, R represents the side chain found in the parent amino acid listed.

The occurrence of didemnins in ascidians belonging to different taxonomic genera such as *Aplidium* and *Tridemnum* suggests these metabolites may at least in part be synthesized by an algal symbiont. Although the presence of blue-green algae has not been reported for *Aplidium albicans*, an *Aplidium* species collected in New Zealand was found to contain several species of cyanobacteria and eukaryotic algae embedded in the tunic matrix and the tunic pockets [38]. Terrestrial, as well as marine, blue-green algae are well known for their cytotoxic peptides [69]. Some of the peptides isolated from free-living cyanobacteria contain structural components present in the didemnins. For example, the *N,O*-dimethyltyrosine unit found in several of the didemnins is also present in majusculamides A and B, two lipodipeptides isolated from the marine blue-green alga *Lyngbya majuscula* Gomont, the alga responsible for sporadic outbreaks of contact dermatitis [43].

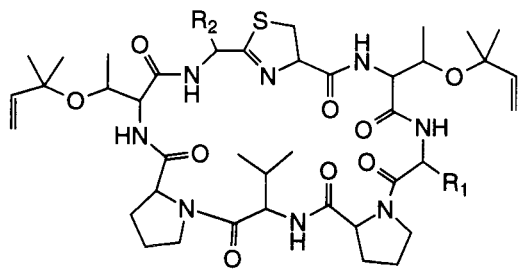
In addition to the didemnins, *T. solidum* has afforded the acyl-tunichlorins, a new class of nickel-containing chlorins (Figure 3) [71]. Acyl-tunichlorins represent the only nickel-containing chlorins to be isolated from a living system and are acyl derivatives of the previously reported tunichlorin [5]. Unique structural features of these compounds include the location and diversity of aliphatic side chains, found at C-2a on the hydroporphyrin nucleus, which are derived from $C_{14:0}$ to $C_{22:6}$ fatty acids. No naturally occurring chlorins with ester-linked acyl side chains at C-2a have been previously reported. Tunicates have been found to contain fixed nickel/cobalt ratios [58], suggesting that these nickel chlorins may play an important metabolic role in *T. solidum*; however, cobalt-containing chlorins have not been detected in *T. solidum*.



patellin 2

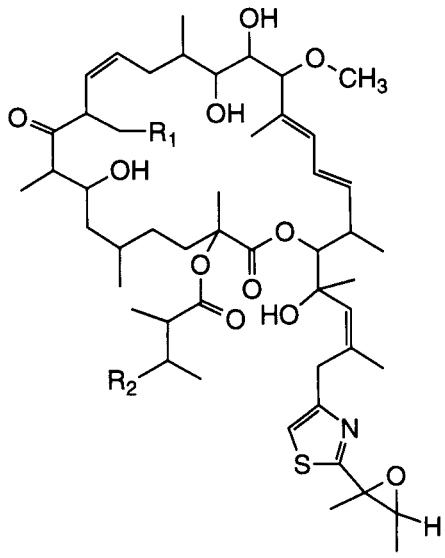


patellin1



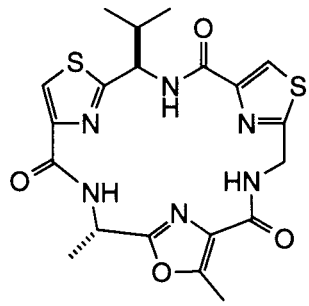
patellin 3 $R_1 = R_2 =$ Leu
patellin 4 R_1 = Leu; R_2=Val
patellin 5 R_1 = Val; R_2= Phe

Figure 7 Patellins 1–5. For amino acid derived metabolites, R represents the side chain found in the parent amino acid listed.



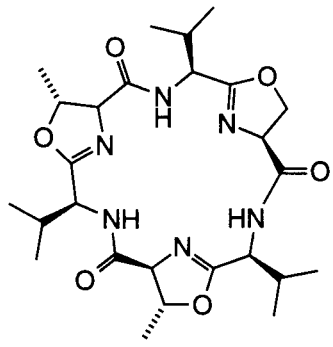
patellazole A $R_1 = R_2 = H$
patellazole B $R_1 = H$; $R_2 = OH$
patellazole C $R_1 = OH$; $R_2 = OH$

Figure 8 Patellazoles A–C.



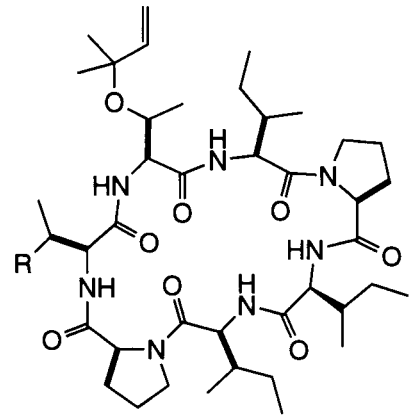
nostocyclamide

Figure 9 Nostocyclamide.



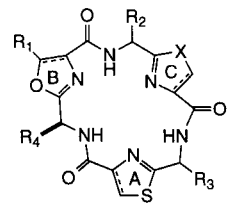
cycloxazoline
(westiellamide)

Figure 10 Cycloxazoline.



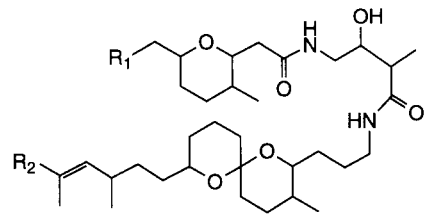
nairaiamide A R = CH_3
nairaiamide B R = CH_2CH_3

Figure 11 Nairaiamides A and B.

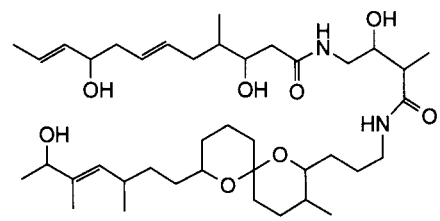


	A	B	C	R^1	R^2	R^3	R^4	X
bistratamide A	thiazoline	oxazoline	thiazoline	CH_3	Ala	Phe	L-Val	S
bistratamide B	thiazole	oxazoline	thiazoline	CH_3	Ala	Phe	L-Val	S
bistratamide C	thiazole	oxazole	thiazole	H	L-Ala	L-Val	L-Val	S
bistratamide D	thiazole	oxazoline	oxazole	CH_3	L-Val	L-Val	L-Val	O

Figure 12 Bistratamides A–D. For amino acid derived metabolites, R represents the side chain found in the parent amino acid listed.

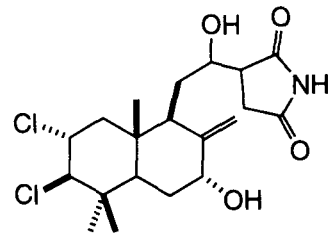


bistramide A $R_1 = COCH{=}CHCH_3$; $R_2 = CHOHCH_3$
bistramide B $R_1 = COCH_2CH_2CH_3$; $R_2 = CHOHCH_3$
bistramide C $R_1 = CH{=}CHCH_3$; $R_2 = COCH_3$
bistramide D $R_1 = CHOHCH{=}CHCH_3$; $R_2 = CHOHCH_3$



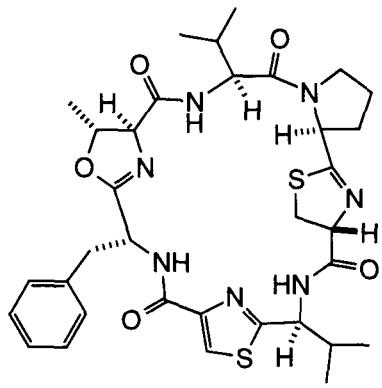
bistramide K

Figure 13 Bistramides A–D, and K.



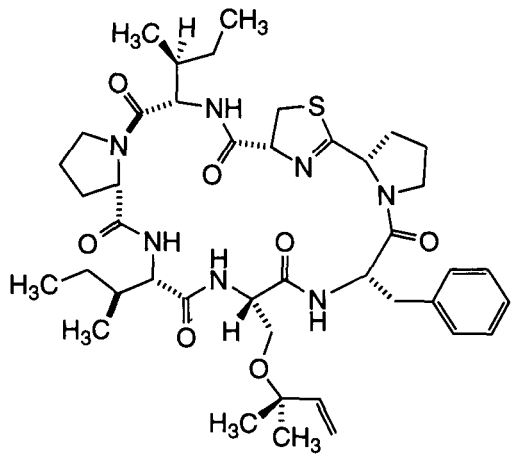
dichlorolissoclimide

Figure 14 Dichlorolissoclimide.



cyclodidemnamide

Figure 15 Cyclodidemnamide.



mollamide

Figure 16 Mollamide.

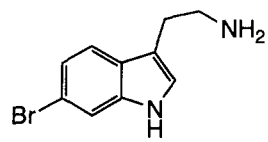
It is reasonable to suggest that the acyl-tunichlorins are tunicate-modified algal products, as ascidians do not contain photosynthetic pigments of their own and no nickel porphynoids have been reported as algal natural products. In a study to ascertain whether the acyl-tunichlorins are produced by the alga or the tunicate, *S. tridemni* cells were isolated from *T. solidum* cells following the protocol of Lewin, and the blue-green pigments of the isolated algal and tunicate cells examined [4]. Acyl-tunichlorins were present in the tunicate-containing fractions, but absent from the isolated alga. On the other hand, chlorophyll a was found exclusively in the algal-containing fraction while pheophytin a was found in both the algal (26%) and tunicate (74%) fractions. From its presence in both fractions, pheophytin a may serve as a biosynthetic precursor of the acyl-tunichlorins. A biosynthetic study is needed to establish whether or not the tunicate utilizes algal pigments to produce the acyl-tunichlorins. Additionally tunichlorin has been isolated from the sea hare *Dolabella auricularia*, an algae-consuming mollusk [55]. The occurrence of this unusual nickel hydroporphynoid in two distinct phyla argues that the algae may play a role in the biosynthesis of these compounds. An examination of the algae consumed by this sea hare would be a fruitful, if difficult, endeavor. It is interesting to note that the only two marine organisms known to produce nickel chlorins also produce potent antitumor cyclic peptides, the dolastatins [54] and the didemnins.

Lissoclinum patella

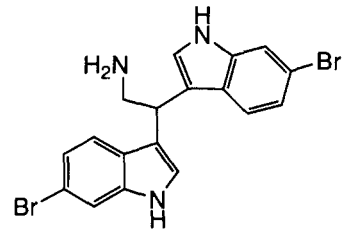
Lissoclinum patella, a large colonial didemnid, is symbiotically associated with *Prochloron* sp. Single colonies are gray-green, may reach 10–25 cm in diameter, be 1–2 cm thick, and weigh up to 200 g. This genus proves advantageous for the study of algal-ascidian symbiosis because it is relatively easy to obtain pure suspensions of *Prochloron* cells by manually pressing the didemnid colony [39]. Suspensions of algal cells are almost free of contaminant microbes, suggesting that the host may favor the sole growth of *Prochloron* [39]. Radiolabeling studies using $^{14}CO_2$ demonstrated that *L. patella* incorporates photosynthate from the associated alga. Colonies incubated in light incorporated 4–5 times as much ^{14}C in the ascidian tissue as dark controls. Most of the ^{14}C transfer from the alga to the host was found in lipids, nucleic acids, protein fractions, and molecules of low molecular weight.

L. patella has provided several families of closely related cyclic peptides: the lissoclinamides (Figure 4) [13,25,27,28,68,77,78], the patellamides (Figure 5) [13,27,28,45,57,66,78] and the tawicyclamides (Figure 6) [44]. The *Lissoclinum* peptides are characterized by the presence of thiazole and/or oxazoline containing moieties. Of these peptides, patellamide D, lissoclinamides 4 and 5, ulithiacyclamide and ascidiacyclamide, were all found within the obligate algal symbiont. The *Prochloron* cells were extracted from the tunicate by slicing the colony horizontally and gently washing the cells with filtered sea water. On a weight to weight basis the amounts of these peptides in *Prochloron* were equal to or greater than the amounts in the host animal alone. Although it is possible that the tunicate could be transferring these peptides to the alga, there are no published accounts on the transfer of organic molecules from an ascidian host to a *Prochloron* cell [39].

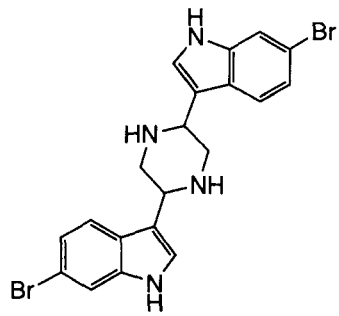
Comparison of the biological activities for these peptides is difficult, as there is little consistency in the types of assays used. Of the patellamides, ulithiacyclamide is the most cytotoxic and gave a T/C value of 178 at 10 mg kg^{-1} against the murine leukemia P1534J cell line and an IC_{50} of 0.35 $\mu g\ ml^{-1}$ for murine leukemia L1210 cells. Ulithiacyclamide B is somewhat more active than ulithiacyclamide against KB cells (IC_{50} = 17 ng ml^{-1} *vs* 35 ng ml^{-1}). For the



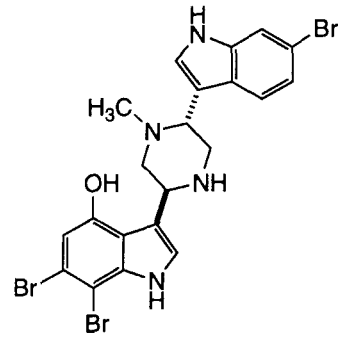
6-bromotryptamine



2, 2-bis(6'-bromo-3'-indolyl)ethylamine



2,5-bis(6'-bromo-3'-indolyl)piperazine



dragmacidin

Figure 17 The *D. candidum* metabolites.

lissoclinamides, it is clear that small structural variations within this family led to dramatic differences in activity. Lissoclinamides 4 and 5 differ only in the oxidation state of a thiazole (thiazoline) unit; however, lissoclinamide 5 is two orders of magnitude less cytotoxic than lissoclinamide 4 against both SV40-transformed fibroblasts (MRC5CV1) and transitional bladder carcinoma (T24) cells. Lissoclinamide 7 is the most cytotoxic, with IC_{50}'s of 0.04 and 0.06 $\mu g\ ml^{-1}$ for a 1-h exposure to MRC5CV1 and T24 cells, respectively.

The tawicyclamides differ from the aforementioned families in that they lack the oxazoline ring characteristic of the patellamides and lissoclinamides. They are also the least cytotoxic, showing equal but weak activity against human colon tumor cells *in vitro*. It appears that the oxazoline ring is essential for the cytotoxicity of this class of compounds, as shown by structure-activity studies [70]. Shioiri and coworkers synthesized ascidiacyclamide, patellamides A–C and ulithiacyclamide, and evaluated them for cytotoxicity against L1210 cells. Their findings show that the oxazoline function is essential for cytotoxicity and even small noncyclic peptides which possess this unit show moderate activity, suggesting a cyclic skeleton is not essential. Ulithiacyclamide, the most potent of these compounds, has a unique disulfide bridge. It appears that this bridge serves to fix the active conformation of the molecule, because cleavage of the disulfide bond results in decreased cytotoxicity. It seems that, although the importance of an oxazoline ring in these peptides cannot be excluded, the overall conformation of the molecule is the most important factor in determining cytoxicity [25].

The patellins are another peptide family isolated from *L. patella* (Figure 7) [11,80]. They are either hexapeptides, patellins 1 and 2, or octapeptides, patellins 3, 4 and 5. These metabolites lack the thiazole and oxazoline amino acids characteristic of the patellamides and lissoclinamides but contain a thiazoline unit. Each compound exists in multiple conformations in solution, which serve to complicate 2D NMR correlation experiments [11]. The structure and relative stereochemistry of the major metabolite, patellin 2, was finally defined using X-ray analysis [80], while the structures of patellins 1, 3, 4 and 5 relied heavily on evaluation of tandem mass spectral data and remain stereochemically undefined [11]. The conformers of patellin 2 were shown to arise from *cis-trans* isomerization of the Val-Pro amide bond, based on NMR and molecular modeling studies. No biological data have been reported for these compounds and the presence of patellins in *Prochloron* has not been investigated.

The presence of a thiazole unit in compounds isolated from *L. patella* is not limited to cyclic peptides. *L. patella* collected in Fiji produced a new family of thiazole-containing polyketide metabolites, the patellazoles (Figure 8) [9,79,81]. They are the most potent cytotoxins to come from a tunicate, with mean IC_{50}'s of 10^{-3}–10^{-6} $\mu g\ ml^{-1}$ in the NCI human cell line protocol. Patellazole B possessed the highest activity, with a mean *in vitro* IC_{50} of less than 10^{-6} $\mu g\ ml^{-1}$ against more than 30 NCI cell lines. Similar

values were obtained against L1210 cells. *In vivo*, patellazole B had no significant antiviral activity in mice infected with HSV-1. Patellazole B has been shown to stimulate incorporation of labeled thymidine into DNA, implying that the compound causes DNA damage. In addition, four minor components, patellazoles D–G, were isolated from the same source. Patellazole F is an isomer of patellazole C, while patellazole G appears to be 31,32-dideoxypatellazole A. The structures of patellazoles D and E have not been assigned [79]. Studies on the biosynthesis of the patellazoles and the role of the algal symbiont in its production have been initiated [9].

For *L. patella*, there are several factors which suggest algal origin for some of the metabolites presented. For example, the types and amounts of cyclic peptides isolated from *L. patella* differ depending on the geographic source of these animals. To cite the patellamides, specimens collected by Ireland *et al* in Eil Malk Island, Palau, yielded patellamides A, B, C, but a specimen collected on the Great Barrier Reef gave patellamide D [68]. On the other hand, *L. patella* collected at Palau Salu, Singapore yielded patellamide E, in addition to A and B [45]. The more recently discovered patellamide F was isolated from an extract of *L. patella* from northwestern Australia. To further confuse the picture, Ireland reported that an *L. patella* specimen collected in Fiji provided no cyclic peptides [13]. There was speculation that the symbiont in this case was a *Synechocystis* species rather than a *Prochloron* [13], arguing that variations in the peptide constituents of *L. patella* could be due to variations in the symbiont. Algal origin is further supported by the fact that some blue-green algae have been known to produce thiazole and oxazoline-containing compounds. Nostocyclamide (Figure 9), recently isolated from a terrestrial blue-green alga, closely resembles the *Lissoclinum* metabolites [73]. The occurrence of macrocyclic thiazole-containing metabolites in unicellular algae independent of didemnids, in conjunction with studies which show that *Prochloron* transfers amino acids to its host [52], implies the metabolic origin of these peptides lies in the *Prochloron*.

Lissoclinum bistratum

L. bistratum is a colonial ascidian which is often colored pink due to the presence of *Prochloron*. As with *L. patella*, the symbiotic interaction between *Prochloron* and *L. bistratum* has been investigated. In a study by Griffiths and Thinh, isolated *Prochloron* was found to transfer 8–34% of photosynthetically fixed $^{14}CO_2$ to the host tissue after a 1-h incubation [21]. The cytotoxic compounds isolated from *L. bistratum* include cycloxazoline (Figure 10) [24], nairaiamides A and B (Figure 11) [17], the bistratamides (Figure 12) [12,16], and the bistramides (Figure 13) [3,18,20].

In terms of symbiosis, cycloxazoline is by far the most interesting metabolite isolated from *L. bistratum*. This compound, reported by the Hawkins group in 1992 [24], is identical to westiellamide, which was isolated from the terrestrial blue-green alga *Westiellopsis prolifica* in the same year by the Moore group [56]. Cycloxazoline was isolated from a methanol-toluene extract of frozen *L. bistratum* colleced from Heron Island Reef, Australia, while westiellamide came from a blue-green alga collected from a mud sample on the island of Oahu, Hawaii. The occurrence of cycloxazoline in a terrestrial cyanophyte suggests that the compound is produced by the *Prochloron* symbiont. Hawkins reported IC_{50} values of 0.5 $\mu g\ ml^{-1}$ against MRC5CV1 and T24 cells for cycloxazoline, while the Moore group reported an IC_{50} of 2 $\mu g\ ml^{-1}$ against KB cells for westiellamide.

Bistratamides A and B, reported earlier by Hawkins, come from the same *L. bistratum*. *Prochloron* cells removed from the host contained large concentrations of these peptides, but they were absent from the *Prochloron*-free ascidian host. Like lissoclinamides 4 and 5, conversion of a thiazoline to a thiazole decreases cytotoxicity ($IC_{50} = 50\ \mu g\ ml^{-1}$ bistratamide A; $IC_{50} > 100\ \mu g\ ml^{-1}$ bistratamide B) for MRC5CV1 and T24 cells. This tunicate also yielded bistratenes A and B, but these compounds could not be detected in the isolated *Prochloron* cells. At the same time, another group reported the isolation of bistramide A. Bistramide A and bistratene A proved to be the same compound, but both groups missassigned the original structure [12,20]. Severe overlap in the proton NMR could not allow for an unambiguous assignment of the structure; however, the correct structure was eventually elucidated in 1992 by Ireland and coworkers using a 2D INADEQUATE experiment optimized for sp^3-sp^3 couplings [18]. The name bistramide A was accepted and bistramides B, C, D and K followed later. Table 1 compares the *in vitro* cytotoxic activities of this family. The pharmacological profile concludes that the presence of an α,β-unsaturated carbonyl group at C-4 (bistratamides A and C) significantly contributes to the observed activity. Bistramides D and K, however, are less toxic *in vivo*, and are thereby more effective as anti-tumor inhibitors in nude mice engrafted with NSCLC-N6.

L. bistratum collected by Ireland in the Philippines lacked all of the metabolites previously described from this organism but contained bistratamides C and D. In addition to the methyloxazoline and thiazole amino acids, bistratamides C and D possess oxazole amino acids, a unit seen in other marine invertebrates including nudibranch egg masses and several sponges [16]. The structural similarity to cycloxazoline/westiellamide suggests these compounds are synthesized by the *Prochloron* cells.

Nairaiamides A and B are two di-proline heptapeptides isolated from a Fijian *L. bistratum* [17]. The nairaiamides

Table 1 Cytotoxic activity of bistramides A, B, C, D, and K against six tumor cell lines (IC_{50} in $\mu g\ ml^{-1}$)[a,b]

Compound	KB	P388	P388/dox[c]	B16	HT29	NCSCL-N6
Bistramide A	0.53	0.20	0.05	0.10	0.32	0.03
Bistramide B	2.10	0.20	1.16	1.20	0.71	0.32
Bistramide C	0.65	0.20	0.05	0.06	0.50	0.05
Bistramide D	10.00	0.36	5.82	0.10	2.76	3.43
Bistramide K	>10.00	0.57	>10.00	1.90	5.60	3.23
6-MP[d]	0.55	0.70	0.26	0.80	0.87	0.79

[a]Adapted from Reference 3.
[b]Mean value for three experiments.
[c]Doxorubicin-resistant.
[d]6-Mercaptopurine as control.

show structural similarity to the patellins, again suggesting *Prochloron* origin. However, no biological data were reported for these compounds and the associated *Prochloron* was not examined.

Lissoclinum voeltzkowi

L. voeltzkowi is a common ascidian which plays an important role in the ecology of sea-grasses [39]. The colonies are gray-green, about 1 mm thick, and up to 30 cm long. Unfortunately, *Prochloron* cells are embedded in the ascidian test, making their removal difficult. Although the tunicate is common, it appears that only one metabolite has been reported from *L. voeltzkowi*. Dichlorolissoclimide (Figure 14) was isolated from the ethanolic extract of *L. voeltzkowi* gathered in New Caledonia [42]. It represents the first labdane diterpene and the first chlorinated compound to be isolated from a tunicate. The succinimide component is also extremely uncommon in naturally occurring compounds. Dichlorolissoclimide is one of the more cytotoxic ascidian compounds to be reported with IC_{50}'s of 14 and 1 ng ml^{-1} against KB and P388 leukemia cells, respectively. The presence or absence of an algal symbiont was not reported.

Didemnum molle

D. molle is the most common and widespread of the obligately symbiotic didemnids. Samples are found on a variety of substrates (dead coral, ropes, shells) and differ greatly in color (white, brown, green, violet) [39]. Although *Prochloron* is incredibly abundant in this tunicate, algal cell preparations cannot be obtained from *D. molle* because the cells are embedded in masses of a mucopolysaccharide. Like *T. solidum*, *D. molle* releases its larvae between 11:00 and 14:00 [49]. The larvae then swim up to 2 h before settling in locations which are optimal for the growth of ascidians and/or algae.

In spite of the presence of *Prochloron*, and the observation that *D. molle* is rarely preyed upon, Fenical has found that this species infrequently contains secondary metabolites [74]. However, our laboratory has found crude extracts of *D. molle* to be cytotoxic against L1210 cells in shipboard assays of samples in Pohnpeii. The lack of secondary metabolites reported from *D. molle* could in part be due to the difficulties associated with the collection of this species. In our experiences, *D. molle* has been a very fragile tunicate which tends to disintegrate after removal from its substrate. To date, only two compounds have been isolated from *D. molle*: cyclodidemnamide (Figure 15) [74] and mollamide (Figure 16) [7]. Mollamide was isolated from *D. molle* collected in Australia. It was screened against a panel of cultured cell lines and shown to be cytotoxic against P388, A549 (human lung carcinoma), HT29, and CV1 (monkey kidney fibroblasts) (IC_{50}'s = 1, 2.5, 2.5 and 2.5 $\mu g\ ml^{-1}$). It also inhibited RNA synthesis with an IC_{50} of ~ 1 $\mu g\ ml^{-1}$. Cyclodidemnamide was isolated from *D. molle* collected in the Philippines. It is weakly cytotoxic against HCT-116 (human colon tumor) cells *in vitro* (ED_{50} = 16 $\mu g\ ml^{-1}$).

Didemnum candidum

D. candidum is an encrusting tunicate which may differ in color, depending on the symbiont [14]. This tunicate has yielded several 6-bromotryptamine derivatives (Figure 17), including 6-bromotryptamine itself, 2,2-bis(6′-bromo-3′-indolyl) ethyl-amine, and 2,5-bis(6′-bromo-3′-indolyl)piperazine. Compounds of this class have been encountered in diverse marine phyla such as sponges [48], tunicates [63] and mollusks [32,33], indicating that these metabolites are produced by symbionts [2,74] or arise from ingested bacteria, as demonstrated by the mollusk *Babylonia japonica* [33]. Algal origin has been further suggested for these *D. candidum* metabolites because dragmacidin, a compound nearly identical to 5-bis(6′-bromo-3′-indolyl)piperazine, was isolated from a deepwater marine sponge, a *Dragmacidon* sp [31].

Conclusions

The occurrence of ascidian metabolites in symbiotic algal cells lends some credence to the argument that some of these metabolites may at least in part be synthesized by the algal symbiont. Furthermore, there are a substantial number of algal metabolites isolated from 'free-living' algae which are similar or identical to ascidian metabolites. A better understanding of the mutual relationships of these organisms, and biosynthetic studies are necessary to firmly establish whether these biologically interesting compounds are produced by the tunicate, the alga, or through a combined effort of both organisms. Clearly the lack of experimental data needed to rigorously establish the true origin of these metabolites does not stem from a lack of interest, but rather from the difficulties encountered when working with these often fragile organisms. The need for establishing metabolic origin is of even greater significance with the advent of ascidian metabolites entering clinical trials. The ability to isolate these metabolites from cultured microorganisms would greatly facilitate pharmaceutical development. For now, however, one should not assume that the tunicate is the sole contributor.

References

1 Aboumansoue E, A Boulanger, A Badre, I Bonnare, B Banaigs, G Combaut and C Francisco. 1995. Tyr (5): didemnin B and D-Pro(4): didemnin B—two new natural didemnins with a modified macrocycle. Tetrahedron 51: 12591–12600.
2 Arillo A, G Bavestrello, B Burlando and M Sara. 1993. Metabolic integration between symbiotic cyanobacteria and sponges: a possible mechanism. Mar Biol 117: 159–162.
3 Baird J-F, C Roussakis, J-M Kornprobst, D Gouiffes-Barbin and J-F Verbist. 1994. Bistramides A, B, C, D, and K: a new class of bioactive cyclic polyethers from *Lissoclinum bistratum*. J Nat Prod 57: 1336–1345.
4 Bible KC. 1989. PhD Thesis. The porphynoid pigments of the Caribbean tunicate *Trididemnum solidum*. University of Illinois at Urbana-Champaign.
5 Bible KC, M Buytendorp, PD Zierath and KL Rinehart Jr. 1988. Tunichlorin: a nickel chlorin isolated from the Caribbean tunicate *Trididemnum solidum*. Proc Natl Acad Sci 85: 4582–4586.
6 Boulanger A, E Abou-Mansour, A Badre, B Banaigs, G Combaut and C Francisco. 1994. The complete spectral assignment of didemnin H, a new constituent of the tunicate *Trididemnum cyanophorum*. Tetrahedron Lett 35: 4345–4348.
7 Carroll AR, BF Bowden, JC Coll, DCR Hockless, BW Skelton and

AH White. 1994. Studies of Australian ascidians. IV* Mollamide, a cytotoxic cyclic heptapeptide from the compound ascidian *Didemnum molle*. Aust J Chem 47: 61–69.
8 Cooper EL. 1985. Immunology: a look toward the sea and what we have learned from tunicates. Aquaculture 132: 1–15.
9 Corley DG and RE Moore. 1988. Patellazole B: a novel cytotoxic thiazole-containing macrolide from the marine tunicate *Lissoclinum patella*. J Am Chem Soc 110: 7920–7922.
10 Crews CM, JL Collins, WS Lane, ML Snappers and SL Schreiber. 1994. GTP-dependent binding of the antiproliferative agent didemnin to elongation factor 1α. J Biol Chem 269: 15411–15414.
11 Davidson BS. 1993. Ascidians: producers of amino acid derived metabolites. Chem Rev 93: 1771–1791.
12 Degnan BM, CJ Hawkins, MF Lavin, EJ McCaffrey, DL Parry and DJ Watters. 1988. Novel cytotoxic compounds from the ascidian *Lissoclinum bistratum*. J Med Chem 32: 1354–1359.
13 Degnan BM, CJ Hawkins, MF Lavin, EJ McCaffrey, DL Parry, AL van den Brenk and DJ Watters. 1989. New cyclic peptides with cytotoxic activity from the ascidian *Lissoclinum patella*. J Med Chem 32: 1349–1354.
14 Fahy E, CM Barbara, D Potts and J Faulkner. 1991. 6-Bromotryptamine derivatives from the Gulf of California tunicate *Didemnum candidum*. J Nat Prod 54: 564–569.
15 Fay P. 1983. Introduction. In: The Blue-greens. p 5, Edward Arnold, London.
16 Foster MP, GP Concepción, GB Caraan and CM Ireland. 1992. Bistratamides C and D. Two new oxazole-containing cyclic hexapeptides isolated from a Philippine *Lissoclinum bistratum* ascidian. J Org Chem 57: 6671–6675.
17 Foster MP and CM Ireland. 1993. Nairaiamides A and B. Two novel di-proline heptapeptides isolated from a Fijian *Lissoclinum bistratum* ascidian. Tetrahedron Lett 34: 2871–2874.
18 Foster MP, CL Mayne, R Dunkel, RJ Pugmire, DM Grant, J-M Kornprobst, J-F Verbist, J-F Baird and CM Ireland. 1992. Revised structure of bistramide A (bistratene A): application of a new program for the automated analysis of 2D INADEQUATE spectra. J Am Chem Soc 114: 1110–1111.
19 Goodbody I. 1974. The physiology of ascidians. Adv Mar Biol 12: 1–149.
20 Gouiffés D, S Moreau, N Helbecque, JL Bernier, JP Hénichart, Y Barbin, D Laurent and JF Verbist. 1988. Proton nuclear magnetic study of bistramide A, a new cytotoxic drug isolated from *Lissoclinum bistratum* Sluiter. Tetrahedron 44: 451–459.
21 Griffiths DJ and L-V Thinh. 1983. Transfer of photosynthetically fixed carbon between the prokaryotic green alga *Prochloron* and its ascidian host. Aust J Mar Fresh Res 34: 431–440.
22 Grubb DR, EJ Wolvetang and A Lawen. 1995. Didemnin B induces cell death by apoptosis: the fastest induction of apoptosis ever described. Biochem Biophys Res Comm 215: 1130–1136.
23 Gutowsky RE. 1984. MS Thesis, University of Illinois, Urbana, IL.
24 Hambley TW, CJ Hawkins, MF Lavin, AL van den Brenk and DJ Watters. 1992. Cycloxazoline: a cytotoxic cyclic hexapeptide from the ascidian *Lissoclinum bistratum*. Tetrahedron 48: 341–348.
25 Hawkins CJ, MF Lavin, KA Marshall, AL van den Brenk and DJ Watters. 1990. Structure-activity relationships of the lissoclinamides: cytotoxic cyclic peptides from the ascidian *Lissoclinum patella*. J Med Chem 33: 1634–1638.
26 Hoffman L and W Greuter. 1993. Validation of *Prochloron didemni* (Cyanophyta) and nomenclatural discussion of correlated names at the higher ranks. Taxon 42: 641–644.
27 Ireland CM, AR Durso, RA Newman and MP Hacker. 1982. Antineoplastic cyclic peptides from the marine tunicate *Lissoclinum patella*. J Org Chem 47: 1807–1811.
28 Ireland CM and PJ Scheuer. 1980. Ulicyclamide and ulithiacyclamide, two new small peptides from a marine tunicate. J Am Chem Soc 102: 5688–5691.
29 Johns RB, PD Nichols, FT Gillan, GJ Perry and JK Volkman. 1981. Lipid composition of a symbiotic Prochlorophyte in relation to its host. Comp Biochem Physiol 69B: 843–849.
30 Kang H, PR Jensen and W Fenical. 1996. Isolation of microbial antibiotics from a marine ascidian of the genus *Didemnum*. J Org Chem 61: 1543–1546.
31 Kohmoto S, Y Kashman, OJ McConnell, KL Rinehart Jr, A Wright and F Koehn. 1988. Dragmacidin, a new cytotoxic bis(indole) alkaloid from a deep water marine sponge, *Dragmacidon* sp. J Org Chem 53: 3116–3118.
32 Kosuge T, K Tsuji, K Hirai and T Fukuyama. 1985. First evidence of toxin production by bacteria in a marine organism. Chem Pharm Bull 33: 3059–3061.
33 Kosuge T, H Zenda, A Ochiai, M Noguchi, S Kimura and H Narita. 1972. Isolation and structure determination of a new marine toxin, surugatoxin from the Japanese ivory shell, *Babylonia Japonica* 26: 2545–2548.
34 Kott P. 1980. Algal-bearing didemnid ascidians in the Indo-west Pacific. Mem Qd Mus 20: 1–47.
35 Kott P. Didemnid-algal symbiosis: host species in the western Pacific with notes on the symbiosis. Micronesia 18: 95–127.
36 Kott P. 1984. Related species of *Tridídemnum* in symbiosis with Cyanophyta. Proc Linn Soc NSW. 107: 515–520.
37 LaFargue F and G Duclaux. 1979. Premier example en Atlantique tropical d'une association symbiotique entre une ascidie didemnidae et une cyanophycee chroococale: *Tridídemnum cyanophorum* nov sp et *Synechocystis tridídemni* nov sp. Ann Inst Oceanogr Paris 55: 163–184.
38 Lambert G, CC Lambert and JR Waaland. 1996. Algal symbionts in the tunics of six New Zealand ascidians (Chordata, Ascidiacea). Invertebrate Biology 115: 67–78.
39 Lewin RA and L Cheng. 1989. Collection and handling of *Prochloron* and its ascidian hosts. In: *Prochloron*: A Microbial Enigma (Lewin RA and L Cheng, eds), pp 9–18, Chapman and Hall, New York, NY.
40 Lewin RA and NW Withers. 1975. Extraordinary pigment composition of prokaryotic algae. Nature (Lond) 256: 735–737.
41 Lindquist N and ME Hay. 1994. Can small rare prey be chemically defended? The case for marine larvae. Ecology 76: 1347–1358.
42 Malochet-Grivois C, P Cotelle, JF Baird, JP Hénichart, C Debitus, C Roussakis and JF Verbist. 1991. Dichlorolissoclimide, a new cytotoxic labdane derivative from *Lissoclinum voeltzkowi* Michaelson (Urochordata). Tetrahedron Lett 32: 6701–6702.
43 Marner F-J and RE Moore. 1977. Majusculamides A and B, two epimeric lidodipeptides from *Lyngbya majuscula* Gomont. J Org Chem 42: 2815–2819.
44 McDonald LA, MP Foster, DR Phillips, CM Ireland, AY Lee and J Clardy. 1992. Tawicyclamides A and B, new cyclic peptides from the ascidian *Lissoclinum patella*: studies of the solution- and solid state conformations. J Org Chem 57: 4616–4624.
45 McDonald LA and CM Ireland. 1992. Patellamide E: a new cyclic peptide from the ascidian *Lissoclinum patella*. J Nat Prod 55: 376–379.
46 Monniot C, F Monnoit and P Laboute. 1991. General organization of an ascidian. In: Coral Reef Ascidians of New Caledonia, pp 9–78, Orstom, Paris.
47 Monniot F. 1980. Present data on metal ions in ascidians. Actual Biochem Mar 2: 185–194.
48 Morris SA and RJ Andersen. 1990. Brominated bis(indole) alkaloids from the marine sponge *Hexadella* sp. Tetrahedron 46: 715–720.
49 Olsen RR. 1983. Ascidian-*Prochloron* symbiosis: the role of larval photoadaptations in midday larval release and settlement. Biol Bull 165: 221–240.
50 Olsen RR. 1986. Photoadaptations of the Caribbean colonial ascidian-cyanophyte symbiosis *Tridídemnum solidum*. Biol Bull 170: 62–74.
51 Paerl HW, RA Lewin and L Cheng. 1984. Variations in chlorophyll and carotenoid pigmentation among *Prochloron* (Prochlorophyta) symbionts in diverse marine ascidians. Botanica Marina 27: 257–264.
52 Pardy RL and RA Lewin. 1981. Colonial ascidians with prochlorophyte symbionts: evidence for translocation of metabolites from alga to host. Bull Mar Sci 31: 817–823.
53 Parry DL. 1984. Cyanophytes with R-phycoerythrins in association with seven species of ascidians from the Great Barrier Reef. Phycologia 23: 503–513.
54 Pettit GR, Y Kamano, CL Herald, AA Tuinman, FE Boettner, H Kizu, JM Schmidt, L Baczynskyj, KB Tomer and RJ Bontems. 1987. Isolation and structure of a remarkable marine animal antineoplastic constituent: dolastatin 10. J Am Chem Soc 109: 6883–6885.
55 Pettit GR, D Kantoci, D Doubek and B Tucker. 1993. Isolation of the nickel-chlorin chelate tunichlorin from the South Pacific ocean sea hare *Dolabella auricularia*. J Nat Prod 56: 1981–1984.
56 Prinsep MR and RE Moore. 1991. Westiellamide, a bistratamide-related cyclic peptide from the blue-green alga *Westillopsis prolifica*. J Nat Prod 55: 140–142.

57 Rashid MA, KR Gustafson, JH Cardellina II and MR Boyd. 1995. Patellamide F, a new cytotoxic cyclic peptide from the colonial ascidian *Lissoclinum patella*. J Nat Prod 58: 594–597.
58 Rayner-Canham GW, M Van Roode and J Burke. 1985. Nickel and cobalt concentrations in the tunicate *Halocynthia pyriformis*: evidence for essentiality of the two metals. Inorg Chim Lett 106: 37–38.
59 Rinehart KL. British Patent Application No. 8922026.3, filed Sept 29, 1989.
60 Rinehart KL. Pharmaceutical compositions containing didemnins. US Patent 5 294.603. Mar 15, 1994: Chem Abstr 1994. 121: 887 m.
61 Rinehart KL Jr, JB Gloer and JC Cook Jr. 1981. Structures of the didemnins, antiviral and cytotoxic depsipeptides from a Caribbean tunicate. J Am Chem Soc 103: 1857–1859.
62 Rinehart KL Jr, JB Gloer, RG Hughes Jr, HE Renis, JP McGovren, EB Swynenberg, DA Stringfellow, SL Kuentzel and LH Li. 1981. Didemnins: antiviral and antitumor depsipeptides from a Caribbean tunicate. Science 212: 933–935.
63 Rinehart KL Jr, J Kobyashi, GC Harbour, RG Hughes, SA Mizak and TA Scahill. 1984. Eudistomins C, E, K, and L, potent antiviral compounds containing a novel oxathiazepine ring from the Caribbean tunicate *Eudistoma olivaceum*. J Am Chem Soc 106: 1524–1526.
64 Sakai R, V Kishore, B Kundu, G Faircloth, JB Gloer, JR Carney, M Namikoshi, F Sun, RG Hughes, DG Gravalos, TG de Quesada, GR Wilson, RM Heid and KL Rinehart. 1996. Structure-activity relationships of the didemnins. J Med Chem 39: 2819–2834.
65 Sakai R, JG Stroh, DW Sullins and KL Rinehart Jr. 1995. Seven new didemnins from the marine tunicate *Tridemnum solidum*. J Am Chem Soc 117: 3734–3748.
66 Schmitz FJ, MB Ksebati, JS Cheng, JL Wang, MB Hossain and D van der Helm. 1989. Cyclic peptides from the ascidian *Lissoclinum patella*: conformational analysis of patellamide D by X-ray analysis and molecular modeling. J Org Chem 54: 3463–3472.
67 Schmitz FJ and T Yasumoto. 1991. The 1990 United States-Japan seminar on bioorganic marine chemistry, meeting report. J Nat Prod 54: 1469–1490.
68 Sesin DF, SJ Gaskell and CM Ireland. 1986. The chemistry of *Lissoclinum patella*. Bull Soc Chim Belg 95: 853–867.
69 Shimizu Y. 1993. Microalgal metabolites. Chem Rev 93: 1685–1698.
70 Shioiri T, Y Hamada, S Kato, M Shibata, Y Kondo, H Nakagawa and K Kohda. 1987. Cytotoxic activity of cyclic peptides of marine origin and derivatives: oxazoline functions. Biochem Pharmacol 36: 4181–4185.
71 Sings HL, KC Bible and KL Rinehart. 1996. Acyl-tunichlorins: a new class of nickel chlorins isolated from the Caribbean tunicate *Trididemnum solidum*. Proc Natl Acad Sci USA 93: 10560–10565.
72 SirDeshpande BV and PL Toogood. 1995. Mechanism of protein synthesis inhibition by didemnin B *in vitro*. Biochemistry 34: 9177–9184.
73 Todorva AK and F Jüttner. 1995. Nostocyclamide: a new macrocycle, thiazole-containing allelochemical from *Nostoc* sp 31 (cyanobacteria). J Org Chem 60: 7891–7895.
74 Toske SG and W Fenical. 1995. Cyclodidemnamide: a new cyclic heptapeptide from the marine ascidian *Didemnum molle*. Tetrahedron Lett 36: 8355–8358.
75 Unson MD, ND Holland and DJ Faulkner. 1994. A brominated secondary metabolite synthesized by the cyanobacterial symbiont of a marine sponge and accumulation of the crystalline metabolite in the sponge tissue. Mar Biol 119: 1–11.
76 Voet D and JG Voet. 1990. In: Biochemistry (Voet D and JG Voet, eds), p 598, John Wiley and Sons, New York.
77 Wasylyk JM, JE Biskupiak, CE Costello and CM Ireland. 1983. Cyclic peptide structures from the tunicate *Lissoclinum patella* by FAB mass spectrometry. J Org Chem 48: 4445–4449.
78 Williams DE and RE Moore. 1989. The structure of ulithiacyclamide B. Antitumor evaluation of cyclic peptides and macrolides from *Lissoclinum patella*. J Nat Prod 52: 732–739.
79 Zabriskie TM. 1989. PhD Thesis. University of Utah.
80 Zabriskie TM, MP Foster, TJ Stout, J Cardy and CM Ireland. 1990. Studies of the solution- and solid-state structure of patellin 2. J Am Chem Soc 112: 8080–8084.
81 Zabriskie TM, CL Mayne and CM Ireland. 1988. Patellazole C: a novel cytotoxic macrolide from *Lissoclinum patella*. J Am Chem Soc 110: 7919–7920.