Ascidians: Producers of Amino Acid Derived Metabolites

Bradley S. Davidson

Department of Chemistry, University of Hawaii, Honolulu, Hawaii 96822

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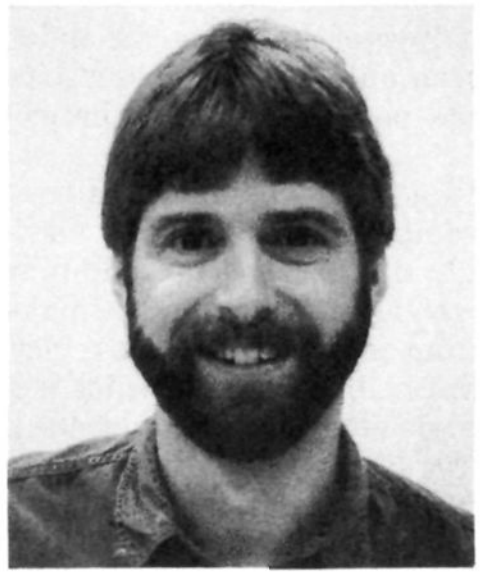
Contents



Bradley Davidson was born in 1959 in Seattle, WA. He obtained a B.S. in chemistry from Montana State University in 1984 and completed his doctorate under the direction of Jerrold Meinwald at Cornell University in 1988. After postdoctoral research with Chris M. Ireland at the University of Utah, he began his current position as Assistant Professor of Chemistry at the University of Hawaii at Manoa. His research interests involve both the isolation/structure determination and synthesis of secondary metabolites from marine invertebrates and microorganisms.

I. Introduction

During the past 25 years, marine natural products chemists have enjoyed unrivaled success in the discovery of unique, new compounds. History has now proven that marine organisms, especially invertebrates such as sponges, soft corals, and molluscs, produce many secondary metabolites which are unprecedented within the terrestrial biosphere. The field of marine natural products is becoming ever more sophisticated. Instead of simply searching for new metabolites, the research has become much more applied, targeting compounds which exhibit pharmacologically useful biological activities. In fact, assay systems have progressed to detect a diverse array of biomedically relevant compounds, including CNS membrane-active toxins, ion channel effectors, anticancer agents, tumor promoters, antiviral compounds, antiinflammatory agents, and metabolites which affect microfilament-mediated processes.[1]

Marine natural products, as a field of scientific endeavor, has grown considerably over the past decade, with approximately 2500 metabolites having been isolated from 1977 to 1987.[1] The expansion of this field of research is most fully appreciated when the above value is compared to the approximately 1700 metabolites reported from marine organisms between the years of 1977 and 1985,[2] indicating that nearly 800 new compounds were isolated during 1987 and 1988. A linear extrapolation of these values would predict that over 4000 compounds will have been reported by the end of 1992, although this may be optimistic considering the inevitable increase in reisolation of known compounds.

Research efforts have not targeted all marine invertebrates equally. The most intensely studied organisms during the decade from 1977 to 1987 were the algae, which supplied investigators with 883 new compounds. Next were the sponges with 736 new compounds, followed by the coelenterates, which yielded 560 new metabolites. In stark contrast, the ascidians were the source of only 65 metabolites during this period.[1]

Studies involving different marine phyla also generate significantly different chemistry.[2] Although most certainly biased by the research interests of individual investigators and the isolation techniques used, each phylum generally affords a characteristic distribution of compound structural types. For example, during the years from 1977 to 1985, 85% of the metabolites isolated from coelenterates were terpenoids, sponges yielded 37% terpenoid and 41% nitrogenous metabolites, and ascidians demonstrated a specialized ability to biosynthesize amino acid derivatives, producing up to 89% nitrogenous compounds.

More recently, while still not commanding the attention reserved for sponges and coelenterates, ascidians have increasingly become the target of natural products research. In fact, between 1988 and mid-1992 an incredible surge of interest in ascidian chemistry yielded approximately 165 new ascidian metabolites, or roughly a 3-fold increase over the previous 10-year time period. These results, which include structurally unprecedented families of biologically activie secondary metabolites, have attracted the attention of both synthetic chemists and pharmacologists.

This review is an attempt to document the exciting new chemistry which has been generated through the

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investigation of marine ascidians. Because the field of marine natural products has been surveyed at relatively frequent intervals,[3] this review does not attempt to discuss all known ascidian metabolites. Instead, only recent results, reported during the years from 1988 through mid-1992, are presented. Furthermore, in contrast to review articles which provide lists of compounds and biological activities, the primary focus of this article will be the structural chemistry of ascidian metabolites. This may include, among other things, methods of structure determination, compound reactivity, and analyses of three-dimensional conformation. There has been an effort to include biological assay results, where possible, but synthetic work is not included.

The overall goal of this article is to illustrate the diversity of compounds produced by marine ascidians, while highlighting their specialized ability to biosynthesize nitrogenous metabolites, the majority of which are derived from amino acids. This review begins with a brief description of ascidians, which is followed by a historical overview to place recent results in perspective. The chemistry of ascidians will be presented in an order based loosely on general compound type and likely biosynthetic origin. Because very little, if any, information is available on the biosynthesis of ascidian metabolites, the categorization was selected for convenience and should in no way be considered as a serious proposal of biosynthetic routes.

II. Organism Description

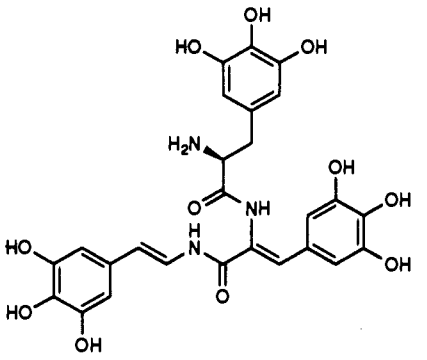
Ascidians belong to the phylum Chordata, which encompasses all vertebrate animals, including mammals. Therefore, they represent the most highly evolved group of animals commonly investigated by marine natural products chemists. Together with the two other classes included in the subphylum Urochordata (= Tunicata), members of the class Ascidiacea are commonly referred to as tunicates, because their body is covered by a saclike case or tunic, or as sea squirts, because many species expel streams of water through a siphon when disturbed. While adult ascidians are exclusively marine invertebrates and bear little resemblance to the other chordates, their larvae resemble amphibian tadpoles and contain notochorods, dorsal hollow nerve cords, and pharyngeal slits, all of which are lost during development.

There are roughly 2000 living species of tunicates,[4] of which ascidians are the most abundant. Adult ascidians are sessile filter feeders, either solitary or colonial, and live preferentially in regions which are free from extensive wave shock, but receive considerable freely flowing sea water. Ascidian morphology is diverse. Solitary tunicates may be up to 15 cm in length, or as small as 1 cm. Colonial species are often found encrusting rocks and may be extremely thin and delicate, or as thick as 5 cm. Some are of indefinate shape, and so disguised by their tunic that they superficially resemble sponges or fleshy coelenterates, yet the contractions which cause a sea squirt to spray streams of water provide the inexperienced observer with a means to distinguish tunicates from other marine invertebrates.

Because a fully expanded specimen is required for taxonomic identification, tunicates must be narcotized before removing them from the water to avoid contractions which will distort the body shape.[5] This may be accomplished by adding a few crystals of menthol to the seawater in the specimen container.[6] After a couple of hours, the specimens may be fixed by adding one-tenth the seawater volume of formalin.

III. Historical Overview

An interest in ascidian chemistry was kindled for much the same reason as many other areas of organic chemistry—*color*. As early as 1847, unusual color changes in ascidian blood were observed upon exposure to air, when the blood would turn from yellow-green to deep blue.[7,8] A German physiologist discovered that the blue coloring was due to relatively large amounts of vanadium and sulfuric acid, along with an uncharacterized nitrogenous metabolite.[9-11] Early researchers proposed that the pigmentation was due to oxygen-carrying proteins called hemovanadins. This postulate has proven wrong, but the conquest to understand the role of vanadium in ascidian blood has led to a tremendous amount of chemistry and biology.[12-14] Ultimately, a series of very unstable hydroquinoid compounds called the tunichromes [e.g. tunichrome An-1 (1)] was isolated from the blood of several species



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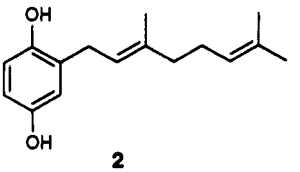
of ascidians.[15,16] While the biological function of these pigments remains unclear, the tunichromes appear to be involved in the sequestration of vanadium by tunicates in concentrations up to 10 million times that found in sea water. Because several recent reviews have been published on the significance of vanadium in ascidians,[13,14] this aspect of the tunichromes will not be elaborated on in this review. However, recent chemical developments will be discussed in the section on peptide metabolites.

Attention has focused, more recently, on ascidians because of their biologically active metabolites. Although the birth of the field of marine natural products is generally credited to Bergmann, who in 1950 isolated several modified arabino nucleosides from a *Tedania* sponge,[17] it was not until 1974 that Fenical isolated the first ascidian metabolite, geranyl hydroquinone (2), from *Aplidium* sp.[18] Compound 2 exhibited chemo-



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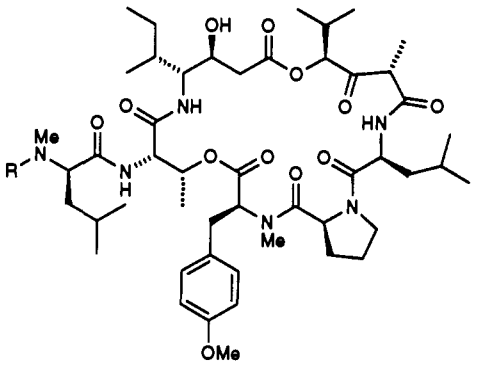
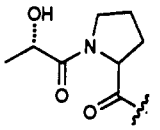
preventive activity against some forms of leukemia, Rous sarcoma, and mammary carcinoma in test animals. The occurrence of an anticancer compound in a marine organism, coupled with the general absence of neoplasms in ascidians, provided a powerful incentive to further expand research on these organisms.

Since that time, ascidians have been the source of numerous natural products, many with interesting biological activities. Thus far, the most successful drug candidate from an ascidian has been the cyclic depsipeptide didemnin B (3), isolated from the Caribbean ascidian *Tridemnum solidum*.[19,20] Didemnin B (3), along with didemnins A (4) and C (5), was proposed to contain the unique structural unit, hydroxyisovaleryl propionate (HIP), and a new *allo* stereoisomer of statine. Later, however, it was determined by synthesis and X-ray crystallography that the residue assigned as statine, was instead isostatine.[21-25]

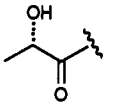


3 R =



4 R = H

5 R =



Perhaps more significant than their unique structures, the didemnins exhibited significant biological activity. The most active didemnin, didemnin B, inhibited DNA viruses *Herpes simplex* types I and II in vitro at 0.05 μM concentrations. Against *Herpes* in mice, didemnin B was able to protect approximately 85% from otherwise lethal vaginal infections.[26,27] Similar results were obtained against the lethal RNA viruses for Rift Valley fever, Venezuelan equine encephalomyelitis, yellow fever, sandfly fever, and a Pichinde virus, at concentrations on the order of 0.05 μg/mL.[28] Mice infected with Rift Valley fever showed 90% survival when treated with didemnin B at 0.25 mg/kg; however, there were some drug related deaths observed at this dose. Didemnin B (3) also has demonstrated in vivo anticancer activity in mice against P388 murine leukemia (T/C 199 at 1.0 mg/kg) and B16 melanoma (T/C 160 at 1.0 mg/kg). Didemnin B was tested against a number of tumors in a human tumor stem cell assay, and significant activity was observed after 1 h of exposure at concentrations as low as 0.1 μg/mL in ovarian, sarcoma, kidney, and breast cells.[29] Recently completed phase II human clinical trials against advanced adenocarcinoma of the kidney,[30] advanced epithelial ovarian cancer,[31] and metastatic breast cancer[32] unfortunately failed to show significant anticancer activity, yet demonstrated significant toxicity. In addition to the extensive list of activities cited above, didemnin B exhibits immunosuppressant activity. In a Simonsen parental-to-F_1 graft-versus-host assay, didemnin B showed 71% inhibition of splenomegally with repetitive doses of 0.3 mg/kg.[33]

IV. Ascidian Metabolites

The metabolites discussed in this review are organized by compound type. The primary division is based on the presence or absence of nitrogen. Nitrogenous metabolites are then further segregated into groups representing structural type (e.g., peptides and polycyclic aromatic alkaloids) or plausible biosynthetic origin (amino acid origin). Because of the small number of non-nitrogenous ascidian metabolites, they are not further divided.

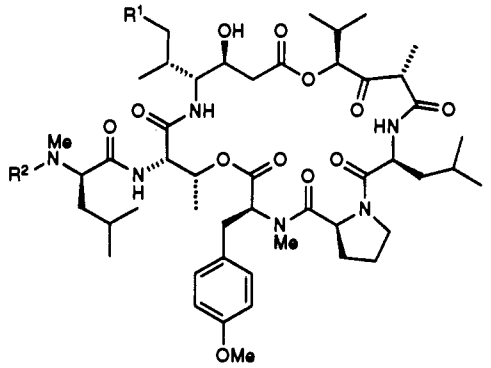
A. Nitrogenous Metabolites

1. Peptide Metabolites

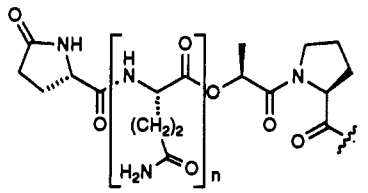
Peptides have continued to be one of the major structural classes isolated from ascidians. Several new didemnins have been reported since the structures of didemnins A (4), B (3), and C (5) were first determined in 1981.[19,20] In addition, several structures which were originally proposed only on the basis of mass spectral data have not been confirmed. Didemnins D (6) and E (7) incorporate the same cyclic depsipeptide structure as didemnins A–C, but their side chains contain three and two contiguous L-glutamines, respectively, and are terminated in a L-pyroglutamate residue.[34] The structure of nordidemnin B (8), which was originally proposed by Gloer based on GC/MS analysis of hydrolysate products,[34] has been confirmed by synthesis and the NMR spectra have been completely assigned.[35,36] Didemnins X (9) and Y (10), which are the most recent additions to the didemnin family of depsipeptides, contain three and four L-glutamine residues, respectively, capped with a new *N*-terminal blocking group, a 3-hydroxydecanoyl unit.[37,38] Several investigations into the solution conformations of didemnin B and related analogs have been carried out.[39-41]

One of the most remarkable features of the didemnins is the variability in biological activity, depending on the acyl substitution pattern.[42] For example, when the side chain is acetyl or longer, the cytotoxic activities are enhanced. A reduction in the cytotoxicity, often without a reduction of antiviral activity, may be accomplished by acylating both the isostatine hydroxyl and the free *N*-methylleucine amino group, or by bridging the amino groups of the *N*-methylleucine and the threonine moieties with a methylene group.[42]

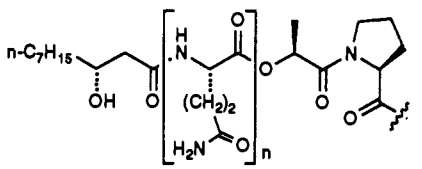
The genus *Lissoclinum* is also a prolific producer of cyclic peptides. Two classes of cyclic peptides, the heptapeptide lissoclinamides and the octapeptide patellamides/ulithiacyclamides,[43] have been isolated from the tunicate *L. patella*. Each of these classes of compounds have been characterized by the presence of



X =



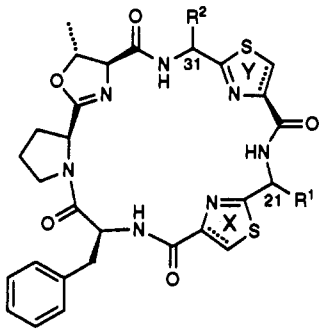
Y =



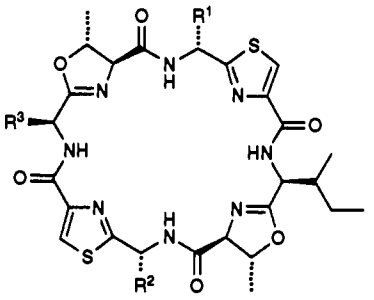
6 $R^1 = CH_3$; $R^2 = X$ (n = 2)
7 $R^1 = CH_3$; $R^2 = X$ (n = 3)
8 $R^1 = H$; R^2 = same as in 3
9 $R^1 = CH_3$; $R^2 = Y$ (n = 3)
10 $R^1 = CH_3$; $R^2 = Y$ (n = 4)

thiazole and oxazoline amino acids. Within the past several years, collections of *L. patella* from a variety of locations have yielded five new octapeptides, lissoclinamides 4 (**11**), 5 (**12**), 6 (**13**), 7 (**14**), and 8 (**15**),[44-46] as well as three new heptapeptides, patellamides D (**16**) and E (**17**)[47] and ulithiacyclamide B (**18**).[48]

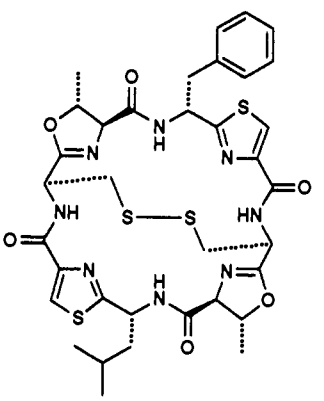
All of the new lissoclinamides **11**–**15** are composed of the same sequence of amino acids. Their differences rest in the oxidation states of the two sulfur-containing rings and in the the absolute stereochemistry of the amino acids. Lissoclinamide 5 (**12**), which contains two thiazole rings, is easily distinguished from lissoclinamide 7 (**14**), which incorporates two thiazoline rings. The remainder of these compounds, lissoclinamides 4 (**11**), 6 (**13**), and 8 (**15**), differ in their biological and chromatographic properties, while containing the same thiazole and thiazoline rings. All of the amino acids in lissoclinamides 4 (**11**) and 6 (**13**) were found to have identical configurations, except for the phenylalanine residues adjacent to the thiazole rings (C21) which were not determined. Compounds **11** and **13** were, therefore, by default, proposed to be C21 epimers. The C21 centers were tentatively assigned as *S* for compound **11** and *R* for **12**, on the basis of ^{13}C NMR chemical shift comparisons with the previously reported ulicyclamide and lissoclinamide 3.[49] Lissoclinamide 8 (**15**) has been very tentatively proposed to have the opposite configurations at C21 and C31 when compared to lissoclinamide 4 (**11**). Most of the lissoclinamides exhibit only mild cytotoxicity. The exceptions are lissoclinolide



11 X = thiazole; Y = thiazoline; R^1 = L-Phe; R^2 = D-Val
12 X = thiazole; Y = thiazole; R^1 = L-Phe; R^2 = D-Val
13 X = thiazole; Y = thiazoline; R^1 = D-Phe; R^2 = D-Val
14 X = thiazoline; Y = thiazoline; R^1 = D-Phe; R^2 = Val
15 X = thiazole; Y = thiazoline; R^1 = D-Phe; R^2 = L-Val



16 R^1 = D-Ala; R^2 = D-Phe; R^3 = L-Ile
17 R^1 = D-Val; R^2 = D-Phe; R^3 = L-Val



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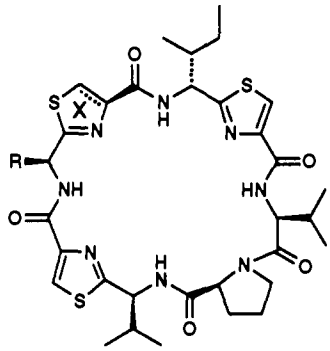
7, which was cytotoxic against MRC5CV1 and T24 cell lines with IC_{50} values of 0.04 μg/mL, and lissoclinamide 4, which was reported active against the same cell lines with an IC_{50} values of 0.8 μg/mL.

Also isolated from collections of *Lissoclinum patella* were ulithiacyclamide B (**18**) and patellamides D (**16**) and E (**17**). Ulithiacyclamide B incorporates a phenylalanine amino acid in place of the alanine found in ulithiacyclamide.[50] Although highly cytotoxic, ulithiacyclamide B, like ulithiacyclamide, showed no selective activity against solid tumor cell lines. An X-ray analysis indicated that patellamide D (**16**) adopts a severely folded conformation with the two thiazole rings nearly parallel to each other.[44] This conformation, which deviates drastically from the nearly square shape reported for the related peptide ascidiacyclamide,[51] is

stabilized by four transannular N–H···O hydrogen bonds. Both structures were similar in that their alkyl groups extend outward; however, whereas patellamide D involves all four NH groups in intramolecular hydrogen bonds, ascidiacyclamide orients all eight nitrogen atoms inward and the oxygen atoms outward, away from the ring.

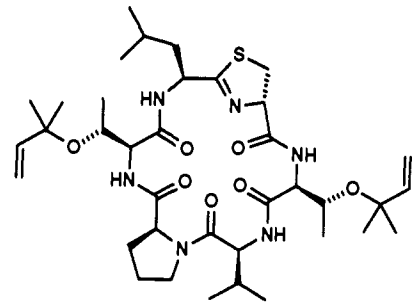
Recently, two new octapeptides, named tawicyclamides A (19) and B (20), were isolated from *L. patella* collected in the Southern Philippines.[52] The tawicyclamides possess one thiazoline and two thiazole amino acids, but lack the oxazoline rings which are characteristic of all previously discovered *Lissoclinum* peptides. The presence of the thiazoline rings was confirmed by oxidation with nickel peroxide to yield the corresponding dehydrotawicyclamides A (21) and B (22), each containing three thiazole rings. X-ray



19 X = thiazoline; R = L-Phe
20 X = thiazoline; R = L-Leu
21 X = thiazole; R = L-Phe
22 X = thiazole; R = L-Leu

crystallographic studies, in combination with molecular mechanics calculations, indicated that tawicyclamide B (20) exists in a three-dimensional conformation very reminiscent to that of patellamide D (16), where the two thiazole rings are essentially parallel to one another. Interestingly, the unusual shape of tawicyclamide B, which was described as having the contour of a "tennis ball seam", underwent an extensive conformational reorientation upon oxidation to dehydrotawicyclamide B. Apparently, the new aromatic thiazole ring both forces the adjacent carbonyl to become coplanar with the ring and induces a *cis*-valine–proline amide linkage to isomerize to *trans*. Together, these changes result in a conformation similar to the "saddle" shape described for ascidiacyclamide.[51] The tawicyclamides and their dehydro analogs exhibited only weak cytotoxicity against human colon tumor cells (IC_{50}'s ~30 μg/mL), which is consistent with structure–activity studies which demonstrated that the oxazoline ring was important for cytotoxicity in a variety of cyclic and acyclic peptides.[53]

A new family of cyclic peptides, called patellins, were isolated from *Lissoclinum patella* collected in the Fiji Islands.[54] The patellins, which include both hexapeptides, patellins 2 (**23**)[55] and 1 (**24**), and octapeptides, patellins 3 (**25**), 4 (**26**), and 5 (**27**), are unique in that they lack the trademark thiazole and oxazoline rings, but contain a thiazoline ring and two novel threonine amino acids which are modified as their dimethylallyl ethers. Each compound exists in solution as a mixture



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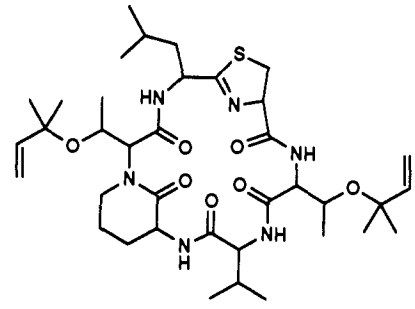

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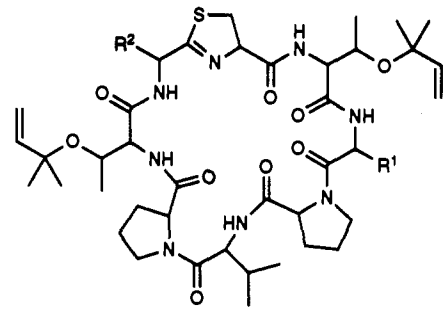


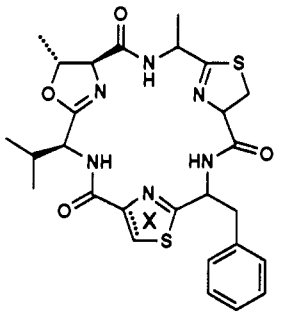
25 $R^1 = R^2$ = Leu
26 R^1 = Leu; R^2 = Val
27 R^1 = Val; R^2 = Phe

of conformers, complicating the interpretation of NMR spectral data. The structure determinations of patellins 1, 3, 4, and 5, therefore, relied heavily on the evaluation of tandem mass spectral data.

Patellin 2 (**23**), the major metabolite, was further investigated using a combination of X-ray crystallography and molecular mechanics calculations.[55] The crystal structure indicated that the peptide was oriented with all of the nonpolar residues on one side, and the thiazoline and many of the carbonyls on the other side. Although the thiazoline ring was shown to exist as two distinct conformers, providing what would appear to be an explanation for the observed doubling of NMR signals, preliminary molecular modeling studies indicated that the two conformers differed in energy by only 0.15 kcal/mol. More importantly, because the barrier to interconversion was only 0.20 kcal/mol above the higher energy conformer, the observed changes in the thiazoline conformation were not sufficient to cause the doubled NMR signals. On the basis of evidence from NMR and molecular modeling, the solution-state conformers were shown, instead, to result from a *cis–trans* isomerization of the valine–proline amide linkage.

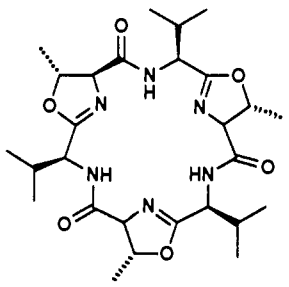
Three new cyclic hexapeptides have also been reported from the related ascidian *Lissoclinum bistratum*,

which was collected from the Great Barrier Reef, Australia. Bistratamides A (28) and B (29)[56] mirror the other *Lissoclinum* peptides by containing both oxazoline and thiazoline rings; however, bistramide A



28 X = thiazole
29 X = thiazoline

contains a second thiazoline ring, whereas bistratamide B has a thiazole ring. Cycloxazoline (30), a symmetrical trimer containing three oxazoline rings formed by the condensation of valine and threonine amino acids,[57]



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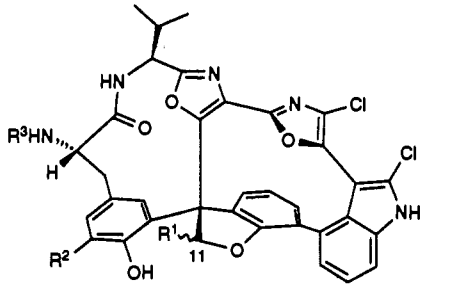
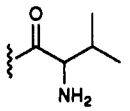
appears to be identical to westiellamide, which was isolated from the terrestrial blue-green alga *Westiellopsis prolifica*.[58] Bistratamides A and B showed only marginal cytotoxicity against the human MRC5CV1 fibroblasts and T24 bladder carcinoma cells with IC_{50}'s in the range of 50 and 100 μg/mL, respectively. Cycloxazoline is cytotoxic agains the same cell lines with IC_{50}'s of 0.5 μg/mL.

The ascidian *Cystodytes dellechiajei* was the source of three cyclic tetrapeptides,[59] each of which was a symmetrical dimer of either L-Leu-L-Pro, L-Leu-L-Val, or L-Pro-L-Phe. These compounds, the structures of which were determined using spectroscopic methods and confirmed by synthesis, were cytotoxic to L1210 leukemia with an IC_{50} of 0.5 μg/mL.

Perhaps the most unusual peptide metabolites isolated from an ascidian are diazonamides A (31) and B (32), which were produced by *Diazona chinensis* collected in the Philippines.[60] These highly unsaturated, chlorinated peptides are made up of derivatives of at least three amino acids: a 3,4,5-trisubstituted L-tyrosine, a tryptophan substituted at the 2- and 4-positions of the indole, and an L-valine. A crystal structure was obtained for the *p*-bromobenzoate derivative of diazonamide B. Derivatization also induced acetal formation between the *p*-hydroxy group of the trisubstituted tyrosine amino acid and the hemiacetal at C11. The UV spectra showed very little indication of the highly unsaturated nature of the diazonamides. Apparently, the high steric requirements of compounds



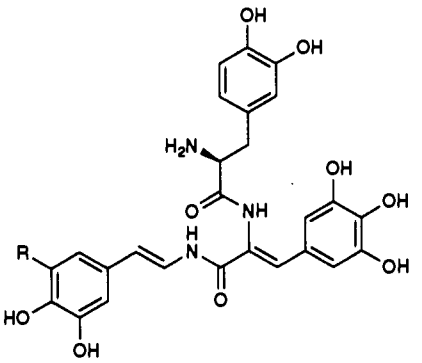
31 R^1 = OH; R^2 = H; R^3 =



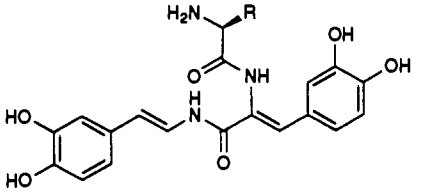
32 R^1 = OH; R^2 = Br; R^3 = H

31 and 32 forces the aromatic rings out of conjugation preventing significant π-overlap. Diazonamide A was cytotoxic in vitro against the human colon cancer HCT-116 and B16 murine melanoma cell lines with IC_{50} values less than 15 ng/mL.

While the vast majority of ascidian metabolites have been isolated from whole-body extractions, several investigations have concentrated on compounds isolated from specific tissues or physiological fluids. The tunichromes, for example, were isolated initially from the blood of the vanadium-sequestering ascidian *Ascidia nigra* and have since been obtained from *Ascidia ceratodes*, *Perophora viridis*, and the iron-accumulating species *Molgula manhattensis*.[14] Within the blood, free tunichromes have been found to residue primarily in the morula cells. Tunichromes An-1 (1), An-2 (33), and An-3 (34) are composed of three DOPA-derived fragments differing in their degree of ring hydroxylation.[15,16] Tunichromes Mm-1 (35) and Mm-2 (36), isolated from



33 R = OH
34 R = H

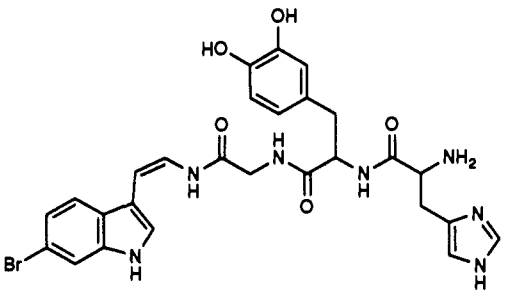


35 R = Gly
36 R = L-Leu

M. manhattensis, bear only two hydroquinoid rings linked to glycine and leucine amino acids, respective-

ly.[16,61] Because these compounds are extremely sensitive to air and water, the original isolation was carried out under totally anaerobic and anhydrous conditions.[15] Synthetic studies have recently yielded a breakthrough in the isolation methodology which involves protection of the hydroxyl groups as their *tert*-butyldimethylsilyl ethers and free amino groups as their *tert*-butyloxycarbonyl derivatives.[62] In addition to allowing the straightforward separation of individual tunichromes, the protecting groups may be subsequently removed, providing native tunichromes for further biological studies.

Two novel linear tetrapeptides, halocyamines A (**37**) and B (**38**), were also isolated from morula blood cells, from the blood of the solitary ascidian *Halocynthia roretzi*.[63,64] The structures are composed of several



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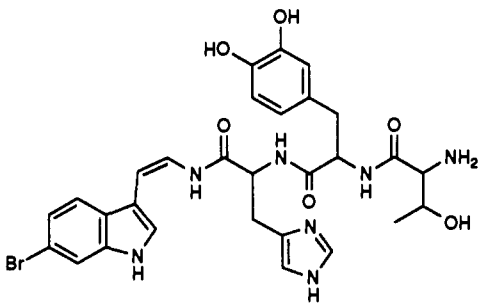
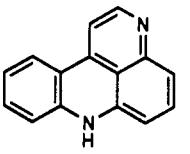


38

amino acids which are unusual to ascidian secondary metabolites, including histidine, 3,4-dihydroxyphenylalanine (DOPA), and 6-bromo-8,9-didehydrotryptamine. The remaining amino acids for halocyamines A and B were glycine and threonine, respectively. The halocyamines possess antiviral activity against fish RNA viruses in RTG-2 cells, as well as antimicrobial activity toward several Gram-positive bacteria and yeasts. They are also cytotoxic to some cultured mammalian cells. Ascidian morula cells have been implicated in immunologic responses such as phagocytosis and lysis of foreign substances. The presence of the halocyamines only in the morula cells of *H. roretzi* may suggest that they play an extracellular role in the defense of this organism.

2. Polycyclic Aromatic Alkaloids

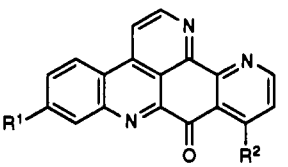
The polycyclic aromatic alkaloids based on the pyrido[*k*,*l*]acridine skeleton (**39**) are, perhaps, the fastest growing class of ascidian metabolites. These compounds, in addition to providing a challenge to natural products chemists, often exhibit an array of



39

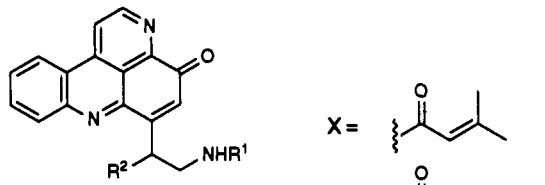
biological activities. Because members of this class of alkaloids are highly unsaturated, leaving very few protons for proton–proton NMR correlations, structural studies have relied heavily on long-range heteronuclear coupling experiments such as COLOC and HMBC. The atom numbering used for the compounds in this section are as assigned by the original authors; therefore, similar substitution patterns are often designated differently.

Ascididemnin (**40**)[65] and 2-bromoleptoclinidinone (**41**),[66,67] from *Leptoclinides* sp. and *Didemnum* sp., respectively, were the first polycyclic aromatic metabolites to be isolated from ascidians. The original structure of **41**, which was assigned based primarily on the results of low power single frequency heteronuclear decoupling experiments, was later revised, using INAPT NMR experiments, to match the carbon skeleton of ascididemin (**40**). Hydrogenolysis of 2-bromoleptoclinidone yielded a compound identical to ascididemin, thus confirming the revised structure. Both compounds were cytotoxic toward leukemia cell lines in vitro with IC_{50}'s of 0.4 μg/mL, while ascididemin also caused the release of calcium in the sarcoplasmic reticulum with a potency 7 times greater than caffeine. More recently, during a reisolation of 2-bromoleptoclinidinone, the related metabolite 11-hydroxyascididemin (**42**) was isolated from *Leptoclinides* sp.[68]



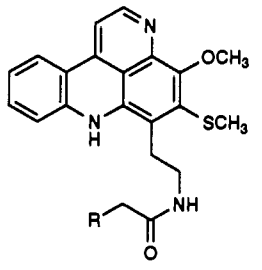
40 $R^1 = R^2 = H$
41 $R^1 = Br$; $R^2 = H$
42 $R^1 = H$; $R^2 = OH$

The cystodytins are a family of nine tetracyclic aromatic alkaloids isolated from the didemnid tunicate *Cystodytes dellechiajei*,[69,70] the same species which produced the three cyclic tetrapeptides described above. Except for cystodytin C (**43**), which bears a 3-hydroxy-3-methylbutyramide moiety, cystodytins were isolated as inseparable pairs of compounds which contain either of two amide substituents. These pairs include cystodytins A (**44**) and B (**45**), D (**46**) and E (**47**), F (**48**) and G (**49**), and H (**50**) and I (**51**). Curiously, each of these pairs were obtained in precisely 3.5:1 ratios. The structure determinations were accomplished primarily using NMR and mass spectrometry, which gave quasi-molecular ions of the form $[M + 2H + H]^+$, as is characteristic of quinones. Like ascididemin, cystodytins A–C showed both cytotoxic activity against L1210 leukemia cells (IC_{50}'s ~0.2 μg/mL) and powerful calcium-releasing activity in sarcoplasmic reticulum. The remaining cystodytins were active against both L1210 cells and human epidermoid carcinoma KB cells with IC_{50} values between 0.068 and 1.4 μg/mL.

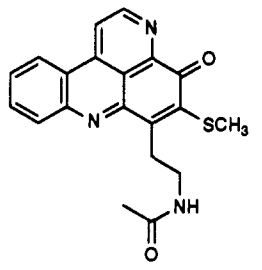


43 R^1 = Z; R^2 = H
44 R^1 = X; R^2 = H
45 R^1 = Y; R^2 = H
46 R^1 = X; R^2 = OH
47 R^1 = Y; R^2 = OH
48 R^1 = X; R^2 = OMe
49 R^1 = Y; R^2 = OMe
50 R^1 = X; R^2 = $OCO(CH_2)_7CH{=}CH(CH_2)_7CH_3$
51 R^1 = Y; R^2 = $OCO(CH_2)_7CH{=}CH(CH_2)_7CH_3$

Three compounds bearing the same carbon skeleton as the cystodytins are varamines A (**52**) and B (**53**)[71] and diplamine (**54**).[72] The varamines are brilliant red pigments which were isolated from a Fijian collection of the ascidian *Lissoclinum vareau*, a genus better known for producing cyclic peptides. Diplamine (**54**) was isolated from another didemnid tunicate of the genus *Diplosoma*. The structure of diplamine was verified by the oxidative demethylation of varamine B (**53**) with ceric ammonium nitrate to give a product identical to diplamine. Varamines A and B and diplamine were all cytotoxic toward L1210 murine leukemia with IC_{50} values of 0.03, 0.05, and 0.02 μg/mL, respectively.

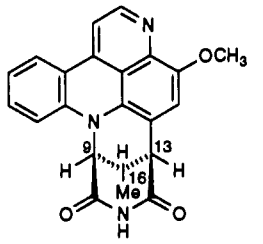


52 R = CH_3
53 R = H



54

A purple tunicate of the genus *Eudistoma* collected in the Red Sea was the source of several novel polycyclic alkaloids.[73,74] A common characteristic trait of the segoline family of compounds was the dramatic color change from orange to deep purple associated with a pH change from neutral to acidic. The structure of segoline A (**55**), which contains an interesting bicyclic imide ring system, was originally deduced using single-crystal X-ray methods. The structure of segoline B (**56**) was subsequently proposed on the basis of spectral studies. NMR showed the ring systems of these two compounds to be identical; therefore, the disparity observed in chromatographic behavior, as well as in the reactivity and biological activity, must be due to stereochemical differences among the three chiral centers. Assuming a *cis* attachment of the glutarimide ring, the difference must either be in the stereochemistry of the two centers attached to the imido ring, or at the lone chiral center bearing the methyl group. The observation of almost mirror-image CD curves for **55** and **56** was interpreted using exciton chirality theory[75] to support an enantiomeric relationship between the two chromophores, thus maintaining the configuration of the lone chiral center, and resulting in an assignment of 9*S**,13*S**,16*R** and 9*R**,13*R**,16*R** for compounds **55** and **56**, respectively.



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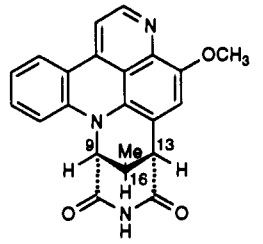
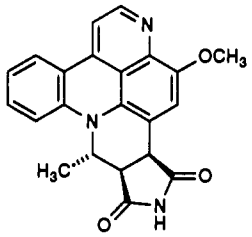


56

The structure of isosegoline (**57**), a more polar isomer of segolines A and B, was proposed on the basis of the interpretation of spectral data.[73,74] While the aromatic ring system proved to be identical to that of **55** and **56**, the imide ring moiety was a succinimide ring rather than a glutaramide ring. The CD spectrum was very similar to that recorded for segoline A, suggesting that both compounds had a similar spatial relationship between the imide and aromatic chromophores. The NMR data obtained for norsegoline (**58**), which was



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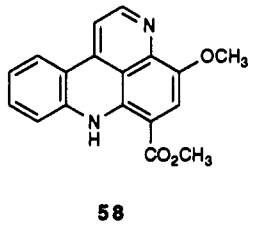
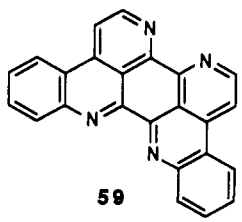


58

only isolated in small amounts, clearly indicated that, while the heteroaromatic moiety was intact, the aliphatic ring system present in the other molecules was entirely absent. In its place, as determined by NOE experiments, was a methoxycarbonyl group. Unfortunately, while these compounds belong to a class of alkaloids often exhibiting biological activity, no assay results were reported.

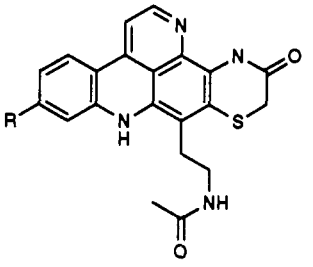
Another interesting compound isolated from the same *Eudistoma* sp. collection was the symmetrical heptacyclic aromatic alkaloid eilatin (**59**).[76] Because this unique, highly symmetrical compound showed only six ^1H and 12 ^{13}C signals in the NMR spectra, complete structural assignment using common spectral methods was impossible, and X-ray crystallography was utilized to determine the final result.



59

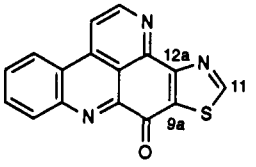
The shermilamines A (**60**)[77] and B (**61**)[78] are thiazinone-containing pentacyclic alkaloids isolated from the

purple tunicate *Tridemnum* sp., collected in Guam. The structure of shermilamine A, which bears a bromine atom at carbon C6, was solved by X-ray diffraction. Only after the subsequent isolation of shermilamine B (6-debromoshermilamine A) and the application of the ^{1}H-detected HMBC experiment were the complete ^{1}H and ^{13}C NMR assignments reported. Shermilamine B was later reported to exhibit cytotoxicity against KB cells with an IC_{50} of 5 μg/mL.[68]



60 R = Br
61 R = H

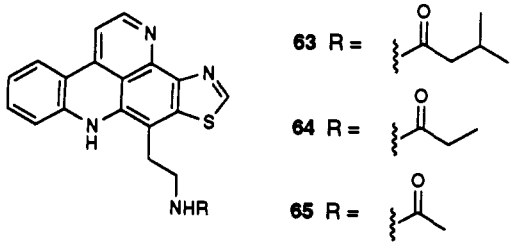
A series of thiazole-containing polycyclic aromatic compounds was isolated from both an unidentified ascidian collected in Pohnpei and the Lamellariidae mollusk *Chelynotus semperi*, believed to be a predator of the ascidian.[79] The structure of kuanoniamine A (**62**), contains a tetracyclic iminoquinone-containing



62

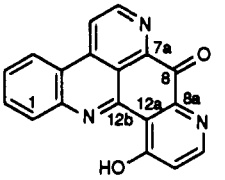
pyridoacridine ring system which is identical to that found in ascididemin (**40**), yet kuanoniamine A incorporates a thiazole ring, whereas **40** contained a pyridine ring. The regiochemistry of the thiazole ring was determined by measuring 3-bond ^{1}H–^{13}C coupling constants between H11–C12a (J = 13.8 Hz) and H11–C9a (J < 5 Hz). The assignment of coupling constants was consistent with several model compounds and was rationalized as resulting from greater electron delocalization across the H11–C11–N–C12a bonds, than across H11–C11–S–C9a. The positioning of sulfur at C9a is also consistent with other ascidian metabolites such as varamines A and B, diplamine, and shermilamines A and B.

Kuanoniamines B (**63**), C (**64**), and D (**65**) contain the same pentacyclic aromatic ring system as kuanoniamine A, but differ in their acylated phenethylamine



side chains.[79] Shermilamine B was also isolated from this ascidian. Cytotoxicities (IC_{50}'s) of the kuanoniamines A, B, and D were 2, >10, and 1 μg/mL against KB cells, respectively.

Meridine (**66**), which was isolated from the ascidian *Amphicarpa meridiana* collected in South Australia,[68] was determined to be a regioisomer of the previously mentioned ascidian metabolite 11-hydroxyascididemin (**42**). Meridine may be formally converted to **42** by moving the C8 carbonyl to a position between C12a and C12b, and connecting C7a to C8a. During a reisolation of this compound using silica gel chromatography, another isomeric metabolite was isolated. While the initial NMR spectra suggested a new compound, after 2 days in $CDCl_3$ the ^{1}H NMR spectrum became indistinguishable from that recorded for meridine. A tautomeric structure **67** was proposed on the basis of NOE experiments. For meridine, dipolar coupling was observed between H1 and an exchangeable proton at δ 15.26 which was assigned to the C12 hydroxyl proton. Alternatively, the spectrum of **67**, lacked the δ 15.26 signal, but showed a correlation between H1 and a new exchangeable proton at δ 12.51, which was assigned to N13–H. Meridine exhibited cytotoxicity against P388 murine leukemia cells at 0.3–0.4 μg/mL.



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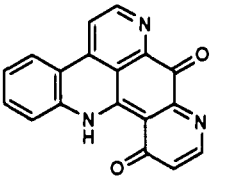
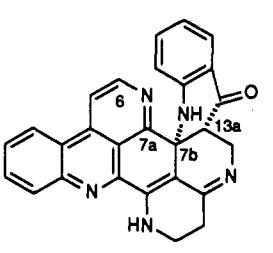


67

Another tunicate of the genus *Eudistoma*, collected in the Seychelles, produced, along with known metabolite ascididemin (**40**), the highly elaborated octacyclic alkaloids eudistones A (**68**) and B (**69**).[80] The structures were proposed based on the interpretation of spectral data. The relative stereochemistry at C7b and C13a was determined from the vicinal ^{1}H–^{13}C coupling constant between H13a and C7a. Heteronuclear proton decoupling of the H6 signal caused the C7a signal to appear as a doublet ($^3J_{H13a,C7a}$ = 1.5 Hz), more closely matching the value predicted from molecular modeling for the *cis*-diequatorial isomer (J = 2.6 Hz) than that calculated for the *trans*-isomer (J = 8.4 Hz). Eudistone B (**69**) was shown to be a dehydrogenation product of **68**, containing two new aromatic NMR signals, in both the ^{1}H and ^{13}C spectra. Furthermore, air oxidation of eudistone A produced a product identical to **69**, thus confirming the relative stereochemistry.



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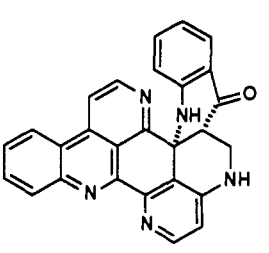
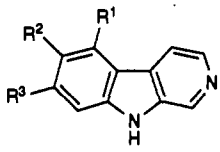


69

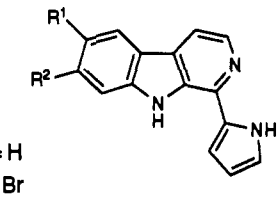
3. Tryptophan-Derived Metabolites

The eudistomins A (**70**) and C–T (**71–88**), the eudistonidins (**91–96**), and woodinine (**97**) make up a large group of tryptophan-derived metabolites based

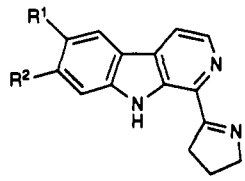
on a β-carboline ring system. The ascidian *Eudistoma olivaceum* has been an extraordinarily rich source of β-carbolines, yielding the eudistomins A (**70**) and C–T (**71–88**),[81–84] several of which exhibited significant



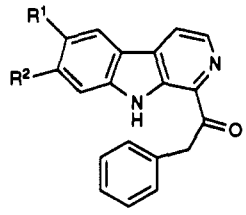
72 R^1 = Br; R^2 = OH; R^3 = H
78 R^1 = H; R^2 = OH; R^3 = Br
82 R^1 = R^3 = H; R^2 = Br
83 R^1 = R^2 = H; R^3 = Br



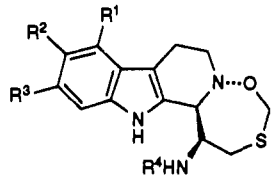
70 R^1 = OH; R^2 = Br
81 R^1 = OH; R^2 = H



75 R^1 = H; R^2 = Br
76 R^1 = Br; R^2 = H
77 R^1 = R^2 = H
84 R^1 = OH; R^2 = Br
85 R^1 = OH; R^2 = H

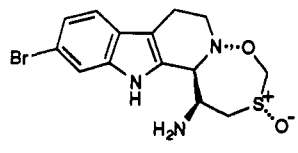


86 R^1 = H; R^2 = Br
87 R^1 = Br; R^2 = H
88 R^1 = R^2 = H



71 R^1 = R^4 = H; R^2 = OH; R^3 = Br
73 R^1 = Br; R^2 = OH; R^3 = R^4 = H
74 R^1 = H; R^2 = OH; R^3 = OH; R^4 = $C_2H_3O_2$
79 R^1 = R^2 = R^4 = H; R^3 = Br
80 R^1 = R^3 = R^4 = H; R^2 = Br
89 R^1 = R^2 = R^3 = R^4 = H

antiviral activity. The eudistomins can be subdivided into the following five classes: the simple β-carbolines [group 1, D (**72**), J (**78**), N (**82**), and O (**83**)], the pyrrolyl-β-carbolines [group 2, A (**70**) and M (**81**)], pyrrolinyl-β-carbolines [group 3, G (**75**), H (**76**), I (**77**), P (**84**), and Q (**85**)], the 2-phenylacetyl-β-carbolines [group 4, R (**86**), S (**87**), and T (**88**)], and the tetrahydro-β-carbolines which contain an oxathiazepine ring [group 5, C (**71**), E (**73**), F (**74**), K (**79**), and L (**80**)]. More recently, known eudistomins C (**71**), K (**79**), and O (**83**), were reported, along with β-carboline and new compounds debromoeudistomin K (**89**) and eudistomin K sulfoxide (**90**), from the New Zealand ascidian *Ritterella sigillinoides*.[85–87] Both NMR and X-ray crystallographic analyses of eudistomin K resulted in a reassigning of the relative stereochemistry of the N–O bond found in the group 5 eudistomins from β to α.[88]



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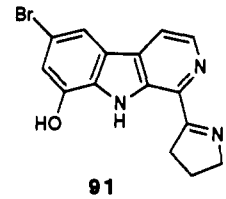
The eudistomidins A–F (**91–96**) and woodinine (**97**) were related β-carboline metabolites isolated from *Eudistoma glaucus*[89–91] and *E. fragum*,[92] respectively. Eudistomidins B (**92**), C (**93**), and D (**94**) showed cytotoxic activity against murine leukemia L1210 (IC_{50} 3.4, 0.36, and 2.4 μg/mL) and L5178Y (IC_{50} 3.1, 0.42, and 2.4 μg/mL) cells, respectively. Eudistomidins A and C also exhibited powerful calmodulin antagonistic activity (IC_{50} = 3 × 10^{-5} M), while B activated actomyosin ATPase in rabbit heart muscle, and D induced the release of Ca^{2+} from the sarcoplasmic reticulum.



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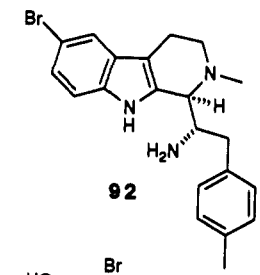

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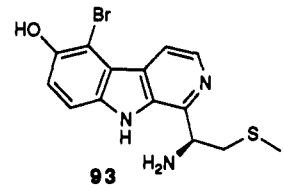

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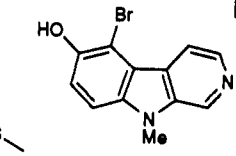

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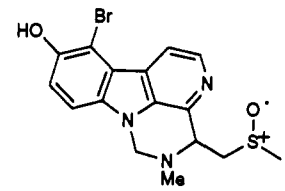

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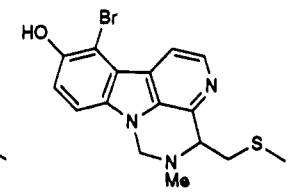

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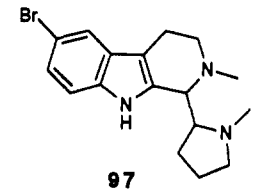
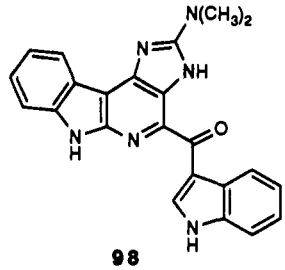


97

All of the β-carboline metabolites isolated thus far are related biosynthetically, being derived from tryptophan and one other amino acid. For example, both group 2 and 3 eudistomins may be considered to be derived from tryptophan and glutamine, while groups 4 and 5 are, in addition to tryptophan, made up of cysteine and phenylalanine or phenylpyruvic acid, respectively. Similarly, eudistomidins B (**92**) and C (**93**) contain units of *p*-methylphenylalanine and *S*-methylcysteine, respectively.

The tunicate *Dendrodoa grossularia* was the source of grossularines 1 (**98**) and 2 (**99**), the first α-carbolines to be isolated from a natural source.[93] Both compounds demonstrated some solid tumor cell selectivity, showing cytotoxicity up to 10 ng/mL against WiDr (colon) and MCF7 (breast) tumor cell lines, but had ID_{50} values of



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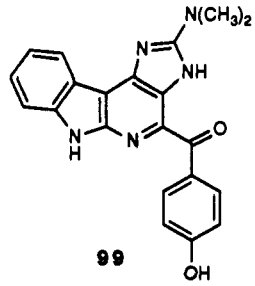
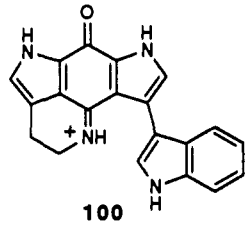


99

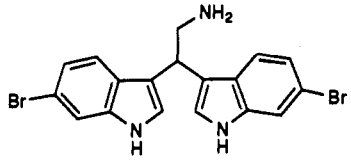
only 4 and 6 μg/mL against L1210 leukemia cells, respectively. Their proposed mode of action includes DNA intercalation.[94]

Wakayin (100) provides the first example of a pyrroloiminoquinone alkaloid to be isolated from an ascidian.[95] Isolated from the Fijian ascidian *Clavelina* sp., wakayin exhibited in vitro cytotoxicity against the human colon tumor cell line HCT-116 with an IC_{50} of 0.5 μg/mL. It also inhibited topoisomerase II at 250 μM and gave a 3-fold differential toxicity toward the CHO cell line EM9 versus BR1, indicating that its cytotoxicity may result from interference with or damage of DNA.



100

Several 6-bromotryptamine derivatives have been isolated from the Gulf of California tunicate *Didemnum candidum*.[96] The structures were assigned as 6-bromotryptamine, 2,2-bis(6′-bromo-3′-indolyl)ethylamine (101), and 2,5-bis(6′-bromo-3′-indolyl)piperazine (102), through interpretation of spectral data. Both 101 and



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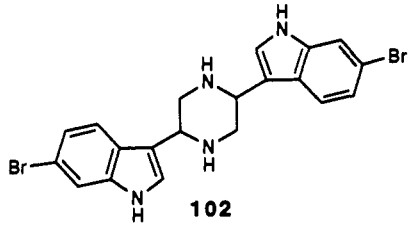
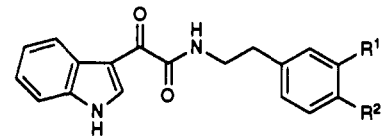


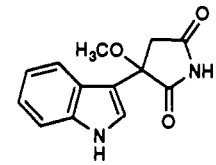
102

102 were found to be optically active. The ^1H NMR spectrum of diacetamide derivative of 102 showed broadened signals due to the interconversion of amide diastereomers, but upon cooling of the sample to −75 °C, the spectrum sharpened, showing sets of equal intensity signals for the three possible diastereomers, containing (*E*,*E*), (*E*,*Z*), and (*Z*,*Z*) amide bonds.

The Philippine ascidian *Polyandrocarpa* sp. was the source of four new metabolites containing derivatives of 4-hydroxyphenethylamine linked to an indole ring through an α-dicarbonyl subunit.[97] The structures of polyandrocarpamides A (103), B (104), C (105), and D (106) were deduced using NMR and mass spectral data. The racemic nature of polyandrocarpamide D raised the possibility that it may be the product of the addition of methanol to an unidentified carbonyl-containing precursor.

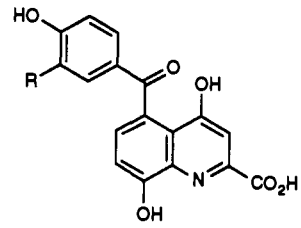


103 R^1 = Br; R^2 = OH
104 R^1 = I; R^2 = OH
105 R^1 = H; R^2 = OH

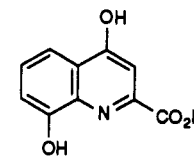


106

An ascidian of the genus *Tridemnum* sp. collected in British Columbia yielded tridemnic acids A (107) and B (108), along with the known metabolite xanthurenic acid (109).[98] During the structure determination of the tridemnic acids, tetramethyltridemnic acid A was brominated in an attempt to prepare a crystalline derivative. Although only a single product was obtained, its structure was unclear. The mass spectrum indicated that three bromine atoms had been introduced, while the ^1H NMR spectrum showed that only two aromatic protons had been replaced. X-ray analysis revealed the unexpected bromination product 110, presumably formed via a novel retro-Fries-type reaction.



107 R = H
108 R = OH



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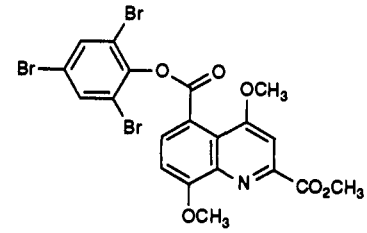
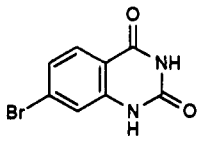


110

Several tunicate metabolites are derived from anthranilic acid, which is commonly obtained from tryptophan. The simple bromoquinazolinedione 111, together with 6-bromoindole-3-carbaldehyde, was iso-

lated from the tunicate *Pyura saccifomis* collected in Japan.[99] The structure, which represents the first brominated quinazolinedione from a natural source, was confirmed by synthesis. The North Sea ascidian *Clavelina lepadiformis* was the source of lepadin A (112), a tetrahydroquinoline alkaloid.[100] Using NOE spectroscopy, the relative stereochemistry was determined to include a *cis* ring fusion, directing both the methyl group and the octa-1,3-diene side chain equatorially and the hydroxyacetyl group axially.



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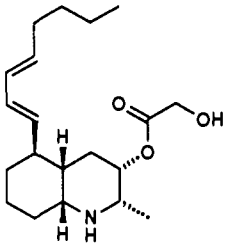
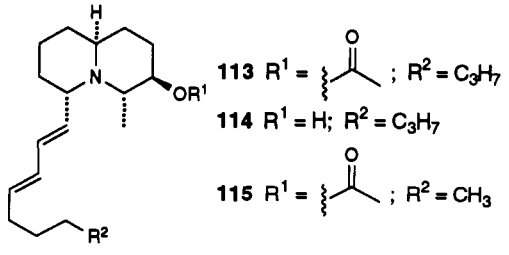


112

4. Lysine-Derived Metabolites

Clavapictines A (113) and B (114)[101] and pictamine (115)[102] are homologous quinolizidine alkaloids isolated from Bermudan and Venezuelan collections of the tunicate *Clavelina picta*, respectively. Mass spectral analysis of hydrogenated clavapictine A revealed a base peak of m/z 210, resulting from the facile loss of a $C_{10}H_{21}$ alkyl side chain. With the use of NOE spectroscopy, 113 was determined to have a *cis* ring junction, with the decadienyl side chain in the equatorial orientation, and the methyl and acetoxy substituents oriented *trans*-diaxial. An X-ray analysis of clavapictine B (114) confirmed the proposed relative stereochemistry and conformation. Apparently, the conformation of the quinolizidine ring system is heavily influenced by the requirement that the alkadienyl side chain lie in the equatorial direction. Also, the alternative *trans* ring fusion is unfavored because it would require a diaxial interaction between the side chain and the methyl group. Pictamine (115) is a bis-nor analog of clavapictine A, bearing two less carbons on the side-chain.

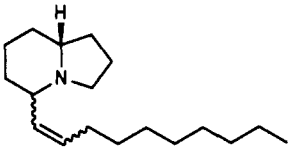


The tunicate *Clavelina picta* has also been the source of a series of indolizine metabolites.[103] The piclavines consist of three groups of compounds, 116–118, each differing in the number of double bonds on the side chain. Each group is composed of an inseparable mixture of stereoisomers, which differ at the C2 chiral center, as well as at each of the double bonds. The piclavine indolizines exhibited antifungal and antimicrobial activity against Gram-positive bacteria (see Table I).

Table I. Antimicrobial Activity of Piclavines A–C (116–118)[a,b]

test organisms	116	117	118
Candida albicans	9	9	6
Geotrichium candidum	12	10	7
Aspergillus tereus	12	10	2
Staphylococcus aureus	4	3	2
Bacillus cereus	5	5	3
Corynebacterium michiganensis	7	6	3

[a] Reference 103. [b] Activity measured as radius of inhibition (mm) from the edge of a disk impregnated with 100 μg of test compound.



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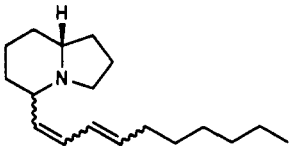

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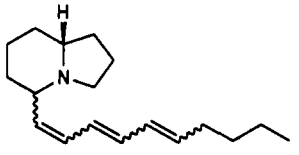
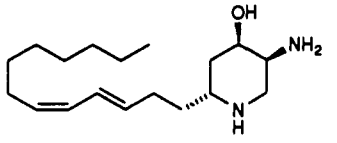


118

The related alkaloids, pseudodistomins A (119) and B (120), were isolated from the Okinawan tunicate *Pseudodistoma kanoko*.[104] Compounds 119 and 120,



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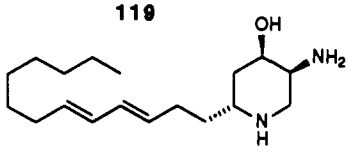
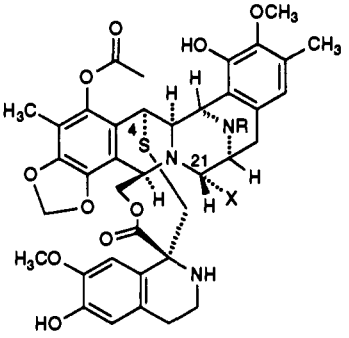


120

which represent the first piperidine alkaloids isolated from a marine source, differ only in the configuration of a double bond. Acetylation of pseudodistomin A provided a derivative which was more stable to air oxidation and double-bond isomerization and was shown to exist as two slowly interconverting conformers. The absolute stereochemistry was determined using the dibenzoate chirality rule of the corresponding dibenzoate substituted at the hydroxyl and primary amino functionalities. Pseudodistomins A and B were cytotoxic, exhibiting IC_{50} values of 2.5 and 0.4 μg/mL against the L1210 and 2.4 and 0.7 μg/mL against the L5178Y cell lines, respectively. In addition, both compounds exhibited inhibitory activity against calmodulin-activated brain phosphodiesterase with an inhibitory concentration of 3×10^{-5} M.

5. Tyrosine- and Phenylalanine-Derived Metabolites

The Caribbean ascidian *Ecteinascidia turbinata* has been of long-standing interest due to reports, as far back as 1969, that aqueous extracts exhibited both in vivo antitumor activity against P388 leukemia and powerful immunomodulatory activity.[105] Now, after 20 years, several interesting tyrosine-derived compounds, called the ecteinascidins, have been isolated independently by two research groups.[106-108] Because of the very small isolated yields, the structure elucidations relied heavily on two-dimensional ^{1}H-detected NMR experiments and FABMS/MS. Detection of the molecular ion of ecteinacidin 743 (**121**), the most abundant metabolite, was hambered by dehydration during FABMS, and only after the application of negative ion FAB mass spectrometry was the correct molecular formula deduced. The lability of the C21 hydroxyl was illustrated by its rapid exchange with methanol or cyanide. The related ecteinascidins 729 (**122**), 745 (**123**), and 770 (**124**) were also isolated, along

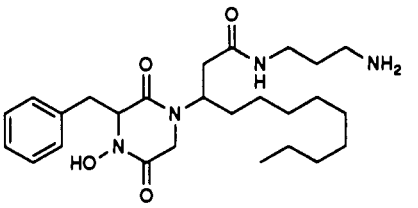


121 R = CH_3; X = OH
122 R = H; X = OH
123 R = CH_3; X = H
124 R = CH_3; X = CN

with 759A and B, which are proposed to be *N*-oxide analogs of **121**. The relative stereochemistry of the C4 chiral center remains ambiguous, with the two research groups indicating opposite configurations. Both papers report that the stereochemistry of the primary ring system is the same as determined for related bacterial metabolites, which include the saframycins[109] and safracins,[110,111] and sponge metabolites, the renieramycins,[112,113] as shown. The ecteinascidins exhibited antitumor activity both in vitro and in vivo: **121**, IC_{50} 0.5 ng/mL against L1210 leukemia cells, T/C 167 at 15 μg/kg against P388 leukemia; **122**, T/C 214 at 3.8 μg/kg against P388 murine leukemia and 246 at 10 μg/kg against B16 melanoma; **123**, IC_{50} 88 ng/mL against L1210 leukemia cells, T/C 111 at 250 μg/kg against P388 murine leukemia.

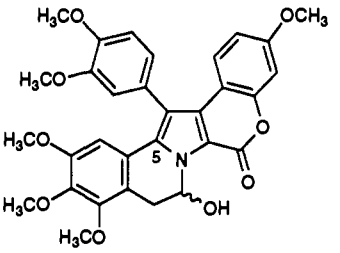
Etzionin (**125**), isolated from an unidentified Red Sea tunicate, is an unusual metabolite which can be dissected into β-aminododecanoic acid, 1,3-diaminopropane, and a 1,4-diketopiperazine modified as the hydroxamate.[114] It exhibited antifungal activity against *Candida albicans* with a MIC of 3 μg/mL, as well as against *Aspergillus nidulans* and the Gram-positive bacterium *Bacillus subtilis*.

Several families of interesting aromatic pyrrole-containing metabolites have been isolated from tunicates in the last 5 years. The first group, the lamellarins,



125

had been isolated previously from the prosobranch mollusc *Lamellaria* sp. (lamellarins A (**126**), B, C, and D),[115] and additional members, lamellarins E (**127**), F (**128**), G (**129**), and H (**130**), have now been isolated



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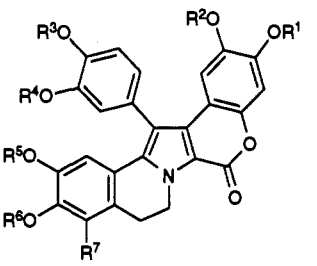
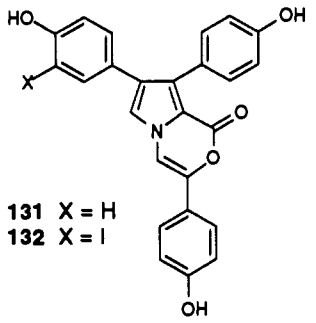


	R^1	R^2	R^3	R^4	R^5	R^6	R^7
127	H	CH_3	CH_3	H	CH_3	CH_3	OH
128	H	CH_3	CH_3	CH_3	CH_3	CH_3	OH
129	CH_3	H	CH_3	H	CH_3	H	H
130	H	H	H	H	H	H	H

from the ascidian *Didemnum chartaceum*.[116] Like the lamellarins A–D, lamellarins E–H are all racemic, each consisting of a pair of atropisomers. Thus far, only lamellarin A has been observed to interconvert between enantiomeric forms. Because molecular mechanics calculations indicated that the barrier to rotation was on the order of 600 kcal/mol, interconversion is, presumably, only possible after opening of the carbinolamine ring. The structures of both lamellarins A (**126**) and E (**127**) were confirmed by X-ray diffraction.

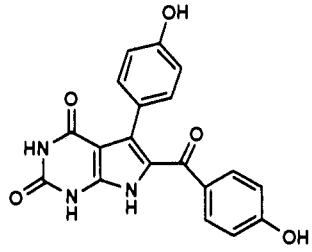
Two related metabolites were recently isolated from an unidentified tunicate from Palmyra Atoll. The structures of the lukianols A and B were determined based on the interpretation of spectral data to be pyrrolooxazinone derivatives **131** and **132**, respectively.[117] Although the relationship is not completely obvious, a formal conversion of a lamellarin into a lukianol can be accomplished by cleavage of the bond between C5 and the aryl substituent in **126**, followed by opening the lactone ring and then reattaching it to the *N*-alkyl chain. Lukianol A exhibited moderate cytotoxicity against KB cells with an MIC of 1 μg/mL.

Rigidin (**133**) is a novel alkaloid isolated from the Okinawan tunicate *Eudistoma* cf. *rigida*,[118] a genus better known for the production of eudistomins and eudistomidins. The pyrrolopyrimidine structure was



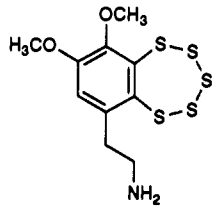
131 X = H
132 X = I

deduced primarily from NMR data. Rigidin exhibited calmodulin antagonistic activity with an IC_{50} value of 5×10^{-5} M for inhibition of calmodulin-activated brain phosphodiesterase.



133

Two unique dopamine-derived polysulfide metabolites were recently isolated from ascidians of the genus *Lissoclinum*. Varacin (134), isolated from *L. vareau*, the same tunicate which produced the varamines, is the first naturally occurring benzopentathiepin.[119] The substitution pattern of the phenyl ring was determined after reductive alkylation gave a *N,N*-dimethylamino derivative bearing two thiomethyl groups, which was analyzed using a combination of NOE and two-dimensional heteronuclear correlation NMR experiments. The presence of the pentathiepin ring, containing five contiguous sulfur atoms, was deduced only by employing negative ion FABMS or EIMS of the *N*-trifluoroacetate derivative. Other methods showed only a strong fragment ion containing three sulfurs. The pentathiepin ring was further confirmed using negative ion FABMS/MS which demonstrated that the pentathiepin molecular ion readily lost S_2. In contrast, lissoclinotoxin A (135), which bears the same substi-



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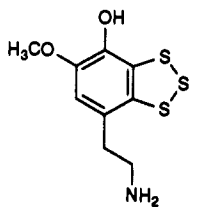
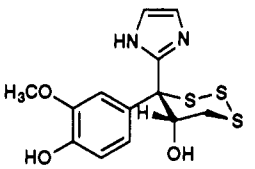


135

tution pattern as varacin, was isolated from the tunicate *L. perforatum* and was proposed to contain a cyclic trithiane ring system.[120] The trithiane ring was deduced by EIMS of the diacetate derivative, while the ring substitution pattern was determined using NOE experiments on a tetraacetate derivative which contained two thioacetate groups. Both compounds were biologically active. Lissoclinotoxin A was reported to exhibit antifungal and antimicrobial activity toward a variety of Gram-positive and Gram-negative bacteria with MIC's in the range of 0.6 to 0.1 μg/mL, as well as against the pathogenic fungi *Candida albicans* (40 μg/mL) and *Trichophyton mentagrophytes* (20 μg/mL). Varacin was active against *C. albicans*, giving a 14-mm zone of inhibition with 2 μg/disk in a disk diffusion assay and cytotoxic toward the human colon cancer HCT-116 cell line with an IC_{90} of 0.05 μg/mL. Furthermore, varacin exhibited a 1.5 differential toxicity toward the CHO cell line EM9 versus BR1, suggesting that varacin damages DNA.

Another trithiane derivative (136) was isolated from the New Zealand ascidian *Aplidium* sp.[121] Compound 136 was observed to be unstable in slightly alkaline solutions or even in neutral CD_3OD, giving a mixture of two new components, one of which was shown to be diastereomer 137, bearing the opposite configuration at the central chiral carbon. The other product was proposed to be thione 138, which upon attempted isolation degraded further to ketone 139.[122] Trithiane 136, therefore, was proposed to be the precursor of 139, a compound which had been previously isolated together with thiazole-containing metabolites 140 and 141 from the Australian ascidian *Aplidium pliciferum*.[123] Conformational analysis of epimer 137 using



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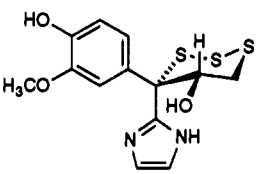

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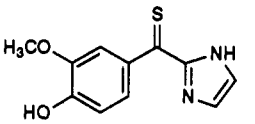

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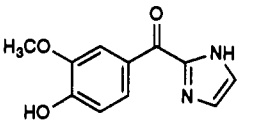

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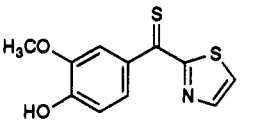

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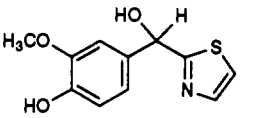
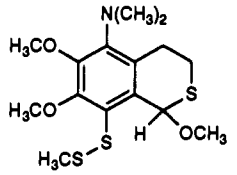


141

NMR and molecular modeling suggested that the trithiane ring prefers a chair conformation with the hydroxyl functionality equatorial and the imidazole ring axial. A similar NMR analysis of natural product 136 was initially interpreted to suggest a skew-boat conformation; however, molecular modeling supported an alternative explanation which included rapid interconversion of the two possible chair conformations, thus giving averaged values for the proton–proton coupling constants. Both trithiane derivatives 136 and 137 exhibited modest cytotoxic activity against P388 murine leukemia with IC_{50} values of 13 and 12 μg/mL, respectively, in addition to inhibiting the growth of *Bacillus subtilis* and *Candida albicans* (~20 μg/disk).

The polycarpamines A–E (142–146), which all incorporate unusual sulfur-containing functional groups, were isolated from a collection of the Philippine ascidian *Polycarpa auzaga*.[124] The presence of these compounds only in the initial collection of *P. auzata*, along with

the lack of optical activity for polycarpamines A, D, and E, made uncertain whether *P. auzata* was the true source, or whether some may be methanol adducts. Polycarpamine B (**143**) exhibited significant antifungal activity against *Saccharomyces cerevisiae* and *Candida albicans*.



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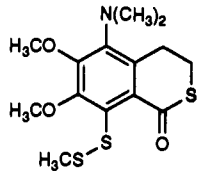

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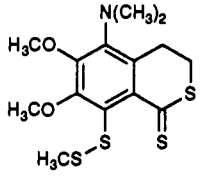

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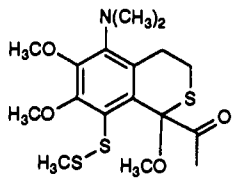

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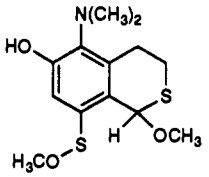
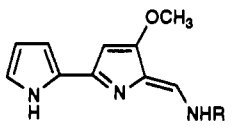


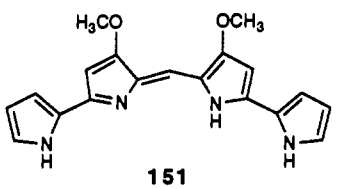
146

6. Miscellaneous Nitrogenous Metabolites

From the ascidian *Atapozoa* sp. collected in the central Philippines, as well as various locations throughout the western Pacific, were isolated two new members of the tambjamine class of alkaloids.[125] Tambjamines E (**147**) and F (**148**) were obtained along with tambjamine C (**149**), a compound previously isolated from the Gulf of California bryozoan *Sessibugula translucens* and its nudibranch predators *Tambje abdere* and *T. eliora*.[126] Interestingly, tambjamines A (**150**), C, E, and F and dimeric tetrapyrrole derivative **151** were also found in nudibranchs of the genus *Nembrotha*, which were commonly observed to feed on *Atapozoa*. Furthermore, the larvae of the ascidian were shown to contain both tambjamine C and tetrapyrrole **151**. Microscopic examination of *Atapozoa* revealed that the bright yellow coloration of the bipyrrole tambjamines was localized within the granular amebocyte blood cells, providing yet another example of ascidian secondary metabolites which are confined to blood cells.

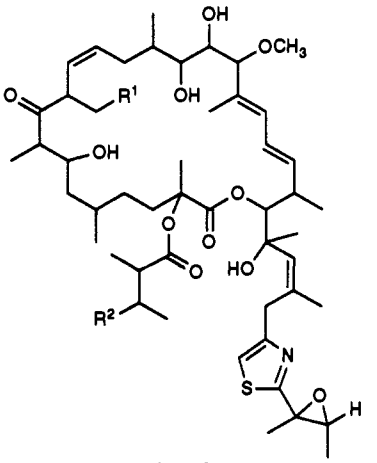


147 R = CH_2CH_3
148 R = CH_2CH_2Ph
149 R = $CH_2CH(CH_3)_2$
150 R = H



151

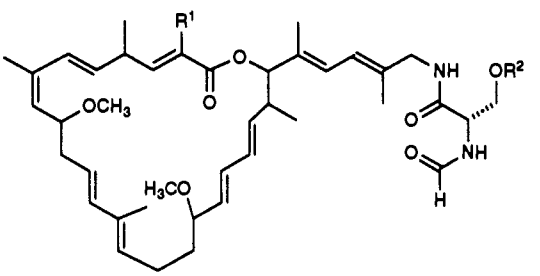
Patellazoles A (**152**), B (**153**), and C (**154**) are cytotoxic thiazole-containing macrolides isolated from the tunicate *Lissoclinum patella*. All three metabolites were obtained from Fijian collections,[127] but patellazole C was also isolated from specimens collected in Guam.[128]



152 $R^1 = R^2 = H$
153 $R^1 = H$; $R^2 = OH$
154 $R^1 = R^2 = OH$

Their structures, which contain a 24-membered macrolide ring, were deduced using an extensive array of two-dimensional NMR experiments, including COSY, TOCSY, RCT1, RCT2, INAPT, COLOC, HMQC, HMBC, and INADEQUATE. While the thiazole ring, a common trademark of *L. patella* metabolites, is most likely amino acid derived, the patellazoles appear to be predominantly of polyketide origin. The patellazoles, which were reported to be potent cytotoxins in the NCI human cell line protocol with mean IC_{50} values of 10^{-3}–10^{-6} μg/mL, also were antifungal against *Candida albicans*. In addition, patellazole C (**154**) exhibited cytotoxicity against the KB cell line with an IC_{50} of 3×10^{-6} μg/mL.

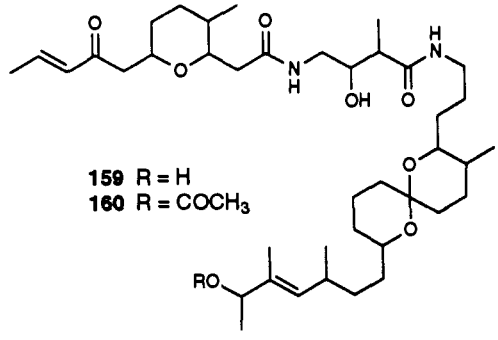
The iejimalides are another family of 24-membered macrolides isolated from the ascidian *Eudistoma* cf. *rigida*,[129,130] the same species that produced rigidin (**133**). Iejimalides C (**155**) and D (**156**) were shown to be sulfate analogs of iejimalides A (**157**) and B (**158**). Iejimalides A and B were cytotoxic toward L1210 (IC_{50} 62 and 32



155 $R^1 = H$; $R^2 = SO_3Na$
156 $R^1 = CH_3$; $R^2 = SO_3Na$
157 $R^1 = R^2 = H$
158 $R^1 = CH_3$; $R^2 = H$

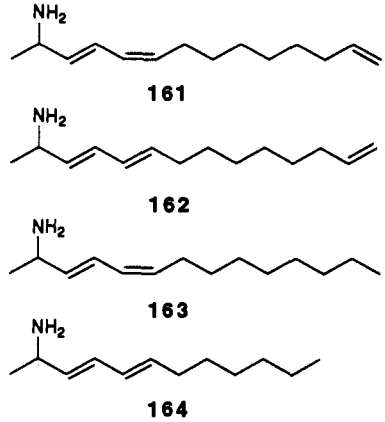
ng/mL) and L5178Y (IC_{50} 22 and 1.0 ng/mL) murine leukemia cells. Iejimalides C and D exhibited significant cytotoxicity against human epidermoid carcinoma KB (IC_{50} 4.7 and 0.2 μg/mL) and L1210 (IC_{50} 10 and 0.58 μg/mL) cells in vitro.

Lissoclinum bistratum, in addition to producing the bistratamide peptides mentioned earlier in this review, also has yielded two polyether metabolites. Several research groups have reported structural information for the compounds which were first called bistramides A and B,[131] and later referred to as bistratenes A and B by another group.[56] Structures proposed for bistramides A and B from these initial studies have now been revised.[132] Severe overlap of signals limited the information available from ^1H NMR experiments and required the use of a two-dimensional INADEQUATE experiment to confirm the linear structure of bistramide A (**159**). HMBC and INAPT experiments were used to establish the correct ether linkages. The related structure, bistramide B, therefore, should bear an acetate on the terminal hydroxyl group as in **160**.

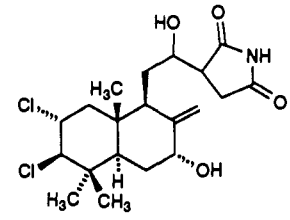


Bistramide A (**159**) has demonstrated extensive activity in a variety of biological assay systems: cytotoxicity toward MRC5CV1 fibroblasts and T24 bladder carcinoma,[133] P388 murine leukemia, KB, and human endothelial carcinoma cell lines,[134] induction of differentiation in HL-60 cells, enhancement of the phospholipid-dependent activity of type II protein kinase C, and induction of blockade in the G1 phase of the cell cycle while causing polyploidy in asynchronous cells of the NSCLCN-L16 cell line.[135]

From the New Zealand ascidian *Pseudodistoma novaezelandiae* were obtained four new amine-containing lipids,[136] similar to the pseudodistomins A (**119**) and B (**120**), which were isolated from another ascidian of the genus *Pseudodistoma*. Two major 14-carbon triene amines **161** and **162**, along with two minor metabolites, a 14-carbon diene amine (**163**) and a 12-carbon diene amine (**164**), proved responsible for the cytotoxic and antifungal activities exhibited by the crude extract. The biological activity of these compounds was proposed to be due to their detergent-like properties.



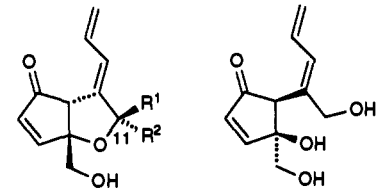
A very unusual compound for an ascidian is the chlorinated labdane derivative dichlorolissoclimide (**165**),[137] which was isolated from *Lissoclinum voeltzkowi*, an organism well known for the production of several non-nitrogenous metabolites, which will be discussed in the next section. Dichlorolissoclimide, which incorporates a rare succinimide moiety, was strongly cytotoxic to human KB cells (IC_{50} 14 ng/mL) and P388 murine leukemia cells (IC_{50} 1 ng/mL).



165

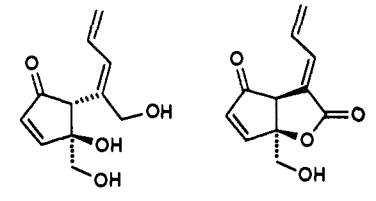
B. Non-Nitrogenous Metabolites

The non-nitrogenous metabolites isolated from ascidians, while few, belong to a variety of structural classes. The metabolites described below are categorized in general structural groups, with some consideration of potential biosynthetic origin. Some interesting non-nitrogenous metabolites which have received considerable attention from synthetic organic chemists are didemnenones A (**166**), B (**167**), C (**168**), and D (**169**).[138-140] Compounds **166** and **167** were isolated from the didemnid tunicate *Trididemnum* cf. *cyanophorum*, collected in the Bahamas, while **168** and **169** were isolated from another didemnid species, *Didemnum voeltzkowi*, which was collected in the Fiji islands, and was previously discussed as the source of dichlorolissoclimide (**165**). Acetals **170** and **171**, which were considered isolation artifacts, could be converted to their corresponding hemiacetals **166** and **167** upon treatment with acid in aqueous THF. The structure



166 R^1 = H; R^2 = OH
167 R^1 = OH; R^1 = H
170 R^1 = H; R^2 = OCH_3
171 R^1 = OCH_3; R^2 = H

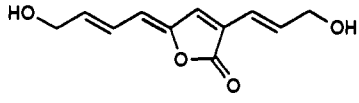
168



169 **172**

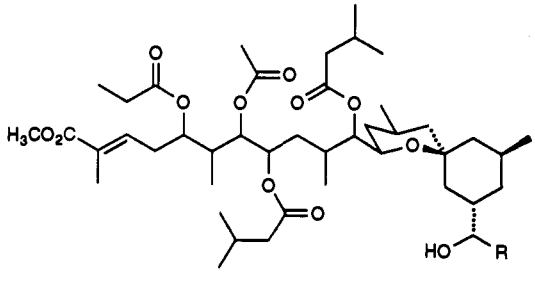
of 170 was confirmed by X-ray analysis. Didemnenones A (166) and B (167) were concluded to be C11 epimers, and not double-bond isomers, on the basis of NOE data. The relative stereochemistry of didemnenones C (168) and D (169) was determined using chemical methods. A MnO_2 oxidation of didemnenone C yielded a lactone product 172, which could also be produced by similar oxidation of 166 and 167. Alternatively, the oxidation product of 169 contained a δ-lactone with a *cis* ring fusion. Surprisingly, the optical rotation of γ-lactone 172 depended on the source organisms. Lactone 172 obtained by oxidation of didemnenones C and D, which came from *D. voeltzkowi*, exhibited a negative rotation, while lactone 172 obtained from didemnenones A and B, isolated from *T. cyanophorum*, had a positive rotation. Didemnenones C and D were cytotoxic toward L1210 murine leukemia cells with IC_{50}'s of 5.6 μg/mL. Didemnenones A and B were active against a variety of microorganisms, including the pathogenic marine fungus *Lagenidium callinectes*.

Lissoclinum patella, which is primarily know as a rich source of thiazole and oxazoline containing cyclic peptides, was also the source of the polyketide-derived lactone lissoclinolide (173).[141] The molecular connectivity was determined primarily through the interpretation of two-dimensional NMR data, while the configuration of the exocyclic double bond was established using NOE data. Lissoclinolide exhibited slight antimicrobial activity against the Gram-negative bacterium *Escherichia coli*.

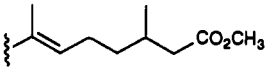


173

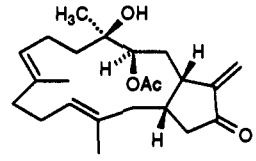
Terpenoid metabolites have seldom been reported from ascidians; however, two unique, highly oxygenated compounds, the didemnaketals A (174) and B (175), were recently isolated from *Didemnum* sp. collected in Palau.[142] Didemnaketal A was suspected to be an oxidation product of didemnaketal B, which has an unusual heptaprenoid structure. Even though both compounds inhibit HIV-1 protease with IC_{50} values of 2 and 10 μM, respectively, the well-established lability of ester functionalities under physiological conditions eliminated chances of further development of these compounds.



174 R = $COCH_3$

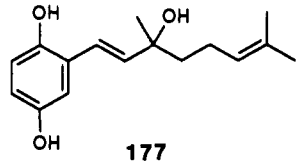
175 R = 

Another metabolite unprecedented from an ascidian is diterpene styelolide (176),[143] which belongs to the cembranoid class of compounds more commonly isolated from soft corals. The ascidian, *Stylena plicata*, was reported to be free from contaminating organisms. The structure was determined by interpretation of two-dimensional NMR data.



176

Two new isoprenoid hydroquinones (177 and 178) and a chromene type metabolite (179), all with antioxidant activity, have been isolated from the colonial tunicate *Amaroucium multiplicatum*.[144] These compounds all were shown to be inhibitory to lipid peroxide formation in rat liver microsomes and to soybean 15-lipoxygenase.



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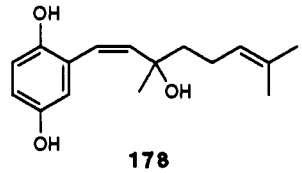

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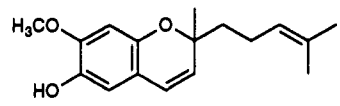
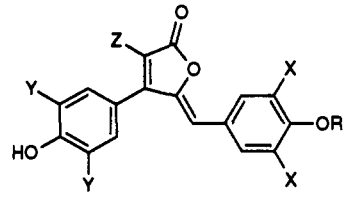
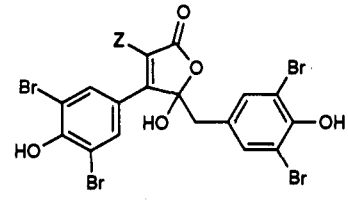


179

The rubrolides A–H (180–187) are a new family of biologically active non-nitrogenous metabolites isolated



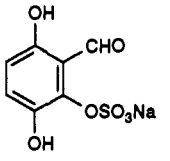
180 R = Z = H; X = Y = Br
181 R = H; X = Y = Br; Z = Cl
182 R = Y = Z = H; X = Br
183 R = X = Z = H; Y = Br
184 R = X = Y = Z = H
185 R = Me; X = Y = Z = H



186 Z = H
187 Z = Cl

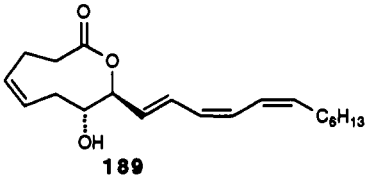
from the colonial ascidian *Ritterella rubra*.[145] Chlorinated ascidian metabolites are rare. While the rubrolides do not contain nitrogen, their similarity to phenylalanine-derived butenolide metabolites isolated from the fungus *Aspergillus terreus*[146] suggests that these compounds may be more closely related to other ascidian metabolites than superficially suspected, possibly originating from phenylalanine or tyrosine. The major rubrolides exhibited the following antimicrobial activities in a disk diffusion assay: *Staphylococcus aureus*, **180** (9 μg/disk), **181** (2 μg/disk), **182** (11 μg/disk); *Bacillus subtilis*, **180** (9 μg/disk), **181** (2 μg/disk), **182** (11 μg/disk). In addition, the rubrolides demonstrated moderate but differential inhibition of protein phosphatases 1 and 2A.

A new sulfated dihydroxylated benzaldehyde compound, polyclinal (**188**), was isolated from the colonial ascidian *Polyclinum planum*, collected near La Jolla, CA.[147] Polyclinal, the structure of which was determined by X-ray crystallography, was found to be most abundant in the zooid-rich surface layer of the colonies.



188

Two families of fatty acid derived lactones have been isolated from didemnid tunicates. Ascidiatrienolides A (**189**), B (**190**), and C (**191**), which contain 9-membered ring lactones, were isolated from the colonial ascidian *Didemnum candidum*.[148] They differ from one another



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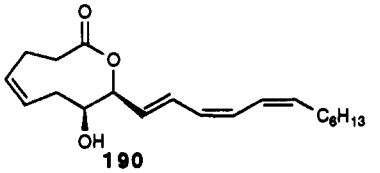

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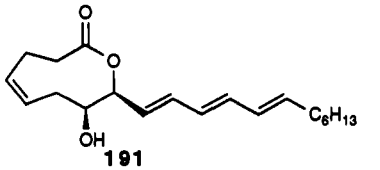

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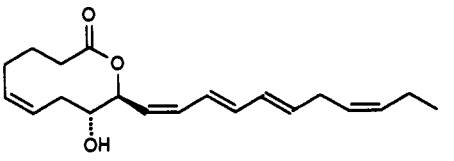
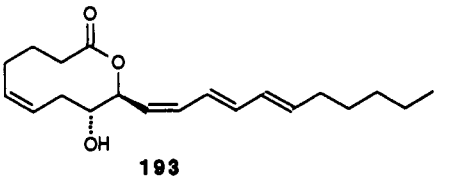
in the configuration of the Δ^{11} double bond, and in the relative stereochemistry of the diol functionality, which was determined on the basis of a comparison of measured $J_{H7,H8}$ coupling constants with those predicted by molecular modeling. The second family includes didemnilactone (**192**) and neodidemnilactone (**193**), both isolated from the tunicate *Didemnum moseleyi*.[149] In contrast to the ascidiatrienolides, **192** and **193** incorporate 10-membered lactone rings. The structures of these compounds were confirmed through synthesis. Together, these metabolites further illustrate the uniqueness of eicosanoid production in marine invertebrates.



192



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V. Conclusion

During the past 5 years, ascidians have been thrust into the limelight of marine natural products chemistry. The increased attention, as illustrated throughout the pages of this review, has resulted in the isolation of numerous new classes of secondary metabolites, many of which exhibit significant biological activities. In fact, as recently as 1987, ascidians had been the source of only some 70 metabolites; whereas, in the years since 1988, nearly 165 new metabolites have been reported! Ascidians remain unique among marine invertebrates in that they overwhelmingly produce nitrogen-containing metabolites, almost all being derived from amino acids. Of the new compounds surveyed in this paper, a vast majority, 83%, contained nitrogen. Although this value is down slightly from the 89% reported by Ireland et al. in 1988,[2] and perhaps biased by the isolation protocols used by individual research groups, the general trend remains strong.

Although research on ascidians was initiated more recently than on some other marine invertebrates, it is significant that the first marine natural product to enter human clinical trials, didemnin B, is an ascidian metabolite. A survey of the new compounds discussed in this review reveals a trend in biological activities. Cytotoxicity is the most frequently listed activity with 68 metabolites reported as active against a variety of tumor cell lines. Next is antimicrobial activity with 22 compounds reported, followed by antiviral with 13, and antiinflammatory with 6 active metabolites. These results, however, are heavily biased, reflecting the selection of assay systems by individual researchers. A more reliable representation of activities would require new metabolites to be tested for a wider array of biomedically important activities.

Our increased understanding of ascidian secondary metabolism has provided a solid foundation for the investigation of several interesting biological aspects of their chemistry. These include whether microalgal symbionts, which are commonly associated with ascidians, play a role in the secondary metabolism, and what purpose these compounds serve for the producing organism in nature. Because the possible ecological roles of marine natural products have been recently reviewed,[150] and these topics will be more fully elaborated on in other articles in this volume, they will be discussed only superficially here.

Ascidians, like many of the other marine invertebrates, are known to exist in obligate and nonobligate symbiosis with microorganisms. Ascidians, in particular, commonly associate with the unicellular prokaryotic alga *Prochloron*,[151,152] as well as with other cyanophytes.[153,154] While the exact nature of the symbiosis is unclear, the increased incidence of algal–tunicate symbioses in low nutrient tropical waters tends to suggest that the algae may play a nutritional role in the host's survival.[155]

For natural products chemists, the most intriguing issue regarding this association is whether the symbionts contribute to the production of secondary metabolites. The isolation of biosynthetically unrelated secondary metabolites from a single organism, which is a rather uncommon event with marine invertebrates, may suggest different origins for each class of compounds. For example, both the lissoclinamides/patellamides and the patellazoles were obtained from *Lissoclinum patella*, an ascidian known to harbor *Prochloron*. Another example involves *Lissoclinum bistratum*, which was the source of the cyclic peptide bistratamides and the polyether metabolites the bistratenes (bistramides). The findings of Hawkins and co-workers, that bistratamides were localized in algal cells, while bistratenes were concentrated in tunicate tissue, are consistent with different origins for these metabolites.[56] Because the symbiotic algae have been resistant to culture, their contributions to the biosynthesis of secondary metabolites have not yet been elucidated. There has been some preliminary success in the mariculture of ascidians, an approach which, presumably, would provide intact symbiont/host pairs for further study.

While an intensive research effort has generated an impressive number of compounds isolated from marine invertebrates, the chemical ecology of these organisms has not received significant attention. This unfortunate fact is not necessarily the result of any lack of interest, but may, instead, be due to the inherent difficulties associated with performing field studies in an aqueous environment. In recent years, primarily because of the imaginative efforts of a small number of researchers, a growing body of experimental data for specific functions of marine products has been presented.

Evidence has been obtained that some secondary metabolites produced by ascidians provide a chemical defense.[156] The tambjamines (**147**–**151**),[157] didemnin B (**3**) and nordidemnin B (**8**), and patellamide C (**152**)[156] have all demonstrated feeding deterrent activities toward generalist fishes in the field. Furthermore, the tambjamines and extracts of the ascidian *Ecteinascidia turbinata*, presumably containing ecteinascidin alkaloids (**121**–**124**),[158] were shown to confer a chemical protection upon their larvae. Eudistomins G (**75**) and H (**76**), extracted from *Eudistoma olivaceum*, were found to inhibit larval settlement, thus reducing surface fouling on the ascidian.[159] While experimental results are beginning to supplant long-standing speculation about the ecological functions of marine secondary metabolites, much research remains to be done.

Considering the increased study of ascidian chemistry, coupled with the relative lack of attention when compared to the sponges and coelenterates, ascidians will undoubtedly continue to be the source of novel secondary metabolites; however, as with other marine invertebrates, the lack of a secure source of large quantities of material will make the development of these compounds as pharmaceuticals difficult. A potential solution of this problem involves the study and culture of symbiotic microorganisms. Increased collaborations between chemists and microbiologists may prove to be a necessary ingredient for future research in marine natural products chemistry.

VI. References

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