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Marine cyanobacteria—a prolific source of natural products

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Contents

1. Introduction

Contemporary trends in drug discovery from natural sources emphasize investigation of the marine environment to yield numerous, often highly complex, chemical compounds.[1–3] Focus on marine biotechnology has been demanded by results indicating that marine microorganisms are substantially involved in the biosynthesis of marine natural products isolated from macroorganisms such as invertebrates.[4,5] In particular, culturable marine microorganisms, notably actinomycetes and fungi, are well known for their production of bioactive metabolites.[6] Cyanobacteria (blue-green algae) have also been identified as one of the most promising groups of organisms from which to isolate novel, biochemically active natural products.[7–10]

Historically, the screening of crude extracts has proven to be an effective method for identifying organisms that produce potentially useful compounds. Early studies, which focused on terrestrial plants and microorganisms, proved extremely fruitful, yielding many useful organic compounds, such as aspirin,® penicillin® and taxol.® By the early 1960s, researchers began to view the oceans as a new and untouched source of potentially useful compounds—perhaps not surprising considering that more than 70% of the Earth's surface is water, with the oceans making up more than 95% of the biosphere. Of the 27 diverse phyla of life, only 17 occur on land, yet all are present in the oceans. Since between 1 and 10% of all marine microbes (including cyanobacteria) are culturable by current techniques, this makes the marine environment one of the last mega-diverse regions of the world yet to be fully studied. By 1975, there were already three parallel tracks of research in marine natural product chemistry: marine toxins, marine bioproducts and marine chemical ecology. Today, marine natural products chemists have determined the chemical structures of over 13,000 novel compounds, and with approximately half of all cancer drug discovery efforts focusing on marine organisms, the forecast for the future looks bright.[11]

The medicinal qualities of cyanobacteria were first

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Table 1. Compounds isolated from marine cyanobacteria (current January 2001). (#) signifies the number of compounds from this particular family of compounds isolated from these marine cyanobacteria and included are known analogues of the major compounds described; a summary of the number of compounds and activities from each order of cyanobacteria, is shown within the table [(#) indicates the total number of species within order or activities, respectively]

Source	Compound	Activity	Class of compound	References
Order chroococcales				
Aphanothece sp.	Poly-3-hydroxyalkanoates	No activity	Alkane	17
Microsystis sp.	Diarrhetic toxin	Cytotoxic	Lipopeptide	18–20
Microcystis aeruginosa	Aeruginosin (**6**), kawaguchipeptin, microcystilide, microcystin (**6**) micropeptin (**3**), microviridin (**2**), toxin BE-4, siatoxin	Enzyme inhibitor, cytotoxic, cell-differentiation, tumor promoter, endotoxic, hepatotoxin, antibiotic, anticancer, toxic	Lipopeptide	15,21–28
M. aeruginosa and *M. wesenburgii*	β-Cyclocitral	No activity	Cyclocitral	29
Microcystis viridis	Cyanoviridin RR	Toxic	Lipopeptide	30,31
Synechococcus sp.	Linolenic acid, phycocyanin	Antibiotic, no activity	Fatty acids, lipopeptide	32
Synechocystis sp.	35-*O*-β-6-Amino-6-deoxyglucopyranosyl bacteriohopanetetrol, nakitriol glycoglycerolipids, nakienone (**3**)	No activity, cytotoxicity	Triterpenoid, fatty acid, C-11 metabolites	33–35
Synechocystis trididemni	Didemnin (**7**)	Anticancer, antiviral, immunosuppressive	Lipopeptide	36–39
Order pleurocapsales				
Hyella caespitose	Carazostatin, chlorohyellazole	Antifungal, no activity	Alkaloids, carbazoles	40
Order oscillatoriales				
Lyngbya bouillonii	Laingolide (**2**), lyngbyaloside lyngbyapeptin, madangolide	Cytotoxic, no activity	Lipopeptide	41–44
Lyngbya gracilis	Azabicyclononane, debromoaplysiatoxin	No activity, cytotoxic	Alkaloids, lipopeptide	45
Lyngbya lagerheimii	Sulfolipid (**4**)	Anti-HIV activity	Fatty acid (sulfo)	46
Lyngbya majuscula	Antillatoxin (**2**), aplysiatoxin (**3**), apramide (**6**), barbamide, γ-lactone (**4**), carmabin (**2**), curacin (**4**), *n*,7-dimethylindole-3-carboxaldehyde, dolastatin (**5**), frontalin, gonyautoxin, grenadadiene (**2**), grenadamide, hermitamide (**2**), kalkipyrone, kalkitoxin, koroamide, laxaphycin (**2**), lyngabellin, lyngbyacarbonate, lyngbyastatin (**4**), lyngbyatoxin (**3**), majusculamide (**6**), maleimide, malyngamide (**26**), malyngic acid, malyngolide (**2**), 7-methoxytetradec-4(*E*)-enoic acid, methoxy-9-methylhexadec-4(*E*)-enoic acid, microcolin (**3**), monoterpene, oscillatoxin, pitamide (**2**), pukelemide (**7**), quinoline (**2**), tanikolide, teleocidin (**5**), ypaoamide, yanacamide (**2**), sulfolipid	Anticancer, antifeedant, antifungal, antiinflammatory, antimicrobial, antimitotic, antiproliferative, antiviral, anti-HIV, brine shrimp toxicity, cytotoxic, cytoskeleton disruption, herbicidal, icthyotoxic, immunosuppressive, molluscidal, neurotoxic, no activity, PBDu binding, tumor promoter, protein kinase activator, skin irritant, toxin	Alkaloids, amide (bromo, chloro and pyrrole), amine, fatty acid (chloro, sulfo and thiazoline), imidazole, lactones, lipopeptides, malyngolide	23,47–74
Oscillatoria sp.	Oscillariolide, diarrhetic toxin	No activity, cytotoxic	Macrolide, lipopeptide	75
Oscillatoria acutissima	Acutiphycin (**21**), (+)-*trans*-20,21-didehydroacutiphycin	Anticancer, cytotoxicity	Ketals, lactones, macrocycle, macrolide	76
Oscillatoria agardhii	Agardhipeptin (**2**), anabaenopeptin (**8**), microcystin (**2**), oscillamide, oscillapeptin (**6**)	Enzyme inhibitor, hepatotoxin	Lipopeptide	77–81
Oscillatoria nigroviridis	Oscillatoxin (**15**)	Toxic general, anticancer	Aromatic, bromo-	82
Oscillatoria raoi	Raocyclamide (**2**), digalactolipids (**4**)	Cytotoxic	Lipopeptide	83
Several *Oscillatoriaceae* sp.	30-Methyloscillatoxin D, 31-noroscillatoxin, oscillatoxin (**3**), tumanoic acid (**5**), scytonemin	Cytotoxic, no activity, sunscreen pigment	Ester, fatty acids, aromatic indoles	53
Phormidium ectocarpi	Hierridin, 2,4-dimethoxy-6-heptadecyl-phenol	Antiplasmodial, antibiotic	Phenol derivatives	84
Phormidium tenue	Sulfolipid (**4**)	Anti-HIV activity	Fatty acid (sulfo)	46,84
Plectonema radiosum	Radiosumin, tubercidin	Enzyme inhibitor	Lipopeptide	85,86

Table 1. (*continued*)

Source	Compound	Activity	Class of compound	References
Schizothrix calcicola	(−)-*E*-1-Chlorotridec-1-ene-6,8-diol, aplysiatoxin (**2**)	Antibiotic, anticancer, cytotoxic	Indole, aromatic, bromo	53,87,88
Spirulina platensis	Calcium spirulan, poly-β-hydroxybutyrate, phycocyanin (**2**)	Anticancer, anti-HIV activity, free-radical scavenger	Saccharides	89–92
Symploca hydnoides	Symplostatin (**2**)	Cytotoxic	Lipopeptide	93
Tridemnum solidum	Acyl tunichlorin	No activity	Nickel chlorins	37
Order nostocales				
Anabaena sp.	Microcystin (**3**), puwainaphycin	Cardioactive, hepatotoxin	Lipopeptide	94,95
Anabaena basta	Bastadin (**20**), bastadin *O*-sulfate esters (**3**)	Antibiotic, antiinflammatory, cytotoxicity	Lipopeptide, esters	96–99
Anabaena circinalis	Circinamide, microcystin	Enzyme inhibitor, toxic	Lipopeptide	100,101
Anabaena flos-aquae	2;9-Diacetyl-9-azabicyclo(4;21)non-2;3-ene, anatoxin (**2**), siatoxin, saxitoxin	Toxic, neurotoxin, antibiotic, anticancer	Lipopeptide, alkaloid	22,24,25,102–105
Anabaena variabilis	Bis(χ-butyrolactones), plastocyanin	Antibiotic, no activity	Lipopeptide	105,106
Anabaena sp.	Diarrhetic toxin	Cytotoxic	Lipopeptide	18–20
Aphanizomenon flos-aquae	Aphanorphine (**2**), siatoxin	No activity, antibiotic, anticancer, toxic	Aromatic, pyrole, lipopeptide	21–23,102
Aulosira fertilissima	Aulosirazole (**2**)	Anticancer	Aromatic	107
Calothrix sp.	Calothrixin (**2**)	Antimalarial, anticancer	Indoles	108
Cyanospira capsulata	4-*O*-[1-Carboxyethyl]mannose, *N*-acetylglucosamine	No activity	Exopolysaccharide	109,110
Cylindrospermum licheniforme	Cylindrocyclophane (**3**)	Anticancer, cytotoxic	Alkaloid, macrocycle, chloro	111
Cylindrospermopsis raciborskii	Cylindrospermopsin (**2**)	Cytotoxic	Alkaloid	112
Hormothamnion enteromorphoides	Hormothamnin (**2**), hormothamnione	Cytotoxicity, antibiotic	Lipopeptide, styrylchromone	113–115
Nodularia sp.	Diarrhetic toxin	Cytotoxic	Lipopeptide	18–20
Nodularia spumigena	Nodulapeptin (**2**), spumigan (**3**)	No activity	Lipopeptide	116
Nodularia harveyana	(3*R*,25*R*)-3,25-Dihydroxygexacosyl-a-D-glucopyranoside	No activity	Glycolipid	117
Nodularia spumigena	ADDA nodularin, nodularia toxin, spumigin, suomilide	Hepatotoxin, enzyme inhibition, toxic, no activity	Lipopeptide, glycoside	118–120
Nostoc sp.	Cryptophycins (**52**), nostophycin, nostocyclamide, nostocyclin, nostoclide (**2**)	Anticancer, cytotoxicity, antifungal, antibiotic, no activity	Amide, lipopeptide	121–123
Nostoc commune	Nostodione, microsporine, diterpenoid (**6**)	Antifungal, antibiotic, antimitotic, cytotoxic, suncreen pigment	Lipopeptide, terpene, oligosaccharide	124–126
Nostoc ellipsosporum	Cyanovirin	Anti-HIV, antiviral	Peptides and proteins	127–129
Nostoc linckia	Borophycin, nostocyclophane	Cytotoxicity	Esters, ketals, pyrans	130,131
Nostoc muscorum	Muscoride	Antibiotic	Lipopeptide	132–134
Nostoc sphaericum	Staurosporine, indolecarbazole (**2**)	Antiviral, cytotoxic	Indoles	135
Nostoc spongiaeforme	Nostocine A, tenuecyclamide (**4**)	Antibiotic, antialgal, cytotoxic, pigment	Lipopeptide	136,137
Rivularia firma	Polybrominated bisindoles (**6**), rivularin	Anti-inflammatory, no activity	Indoles, bromo	138
Scytonema mirabile	Didehydromirabazole (**2**), isonitrile, mirabazole (**2**), tantazole, thiangazole	Antibiotic, cytotoxicity, no activity	Alkaloids	86,139–142
Scytonema pseudohofmanni	Halichondrin, scytophycin, swinholide	Cytotoxic, antifungal, antiviral, antimitotic	Lipopeptide	86,139,143
Tolypothrix sp.	Kalkipyrone	Cytotoxicity	γ-pyrone	51
Tolypothrix conglutinata	Nonamethoxy-1-pentacosene, polymethoxy-1-alkenes (**7**), scytophycin, tolytoxin	Cytotoxicity, anticancer, no activity	1,3-Polyols, isotactic alkenes, lactones	139,144–146
Tolypothrix nodosa	Tolyporphin (**2**)	Antibiotic	Pyrrole	147
Tolypothrix tenuis	Toyocamycin, tubercidin	Antifungal, cytotoxic	Nucleosides	146
Order stigonematales				
Fischerella muscicola	Fischerellin	Antifungal, herbicidal	Lipopeptide	148,149
Hapalosiphon fontinalis	Anhydrohapaloxindole, fontonamide, hapalindole (**5**)	Antibiotic, antifungal	Alkaloids, indoles	150
Hapalosiphon welwitschii	Hapalosin, welwistatin, welwitindolinone	Anticancer, antibiotic, antimitotic, cytotoxic	Alkaloids, lipopeptide	151,152
Prochlorothrix hollandica	Triterpenoids (**3**)	No activity	Terpenes (hopanes)	153
Stigonema dendroideum	Dendroamide	Cytotoxicity, antibiotic	Lipopeptide	154
Westiellopsis prolificans	Westiellamide	Cytotoxicity	Lipopeptide	147

Table 1. (*continued*)

Order	Compounds	Activities
Chroococcales (**11**)	36	Enzyme inhibitor, cytotoxic, cell-differentiation, tumor promoter, endotoxic, hepatotoxic (**6**)
Pleurocapsales (**1**)	2	Antifungal, no activity (**2**)
Oscillatoriales (**15**)	197	Antialgal, anticancer, anti-HIV, antifeedant, antifungal, anti-inflammatory, antimicrobial, antimitotic, antiproliferative, antiviral, brine shrimp toxicity, cytotoxic, cytoskeleton disruption, herbicidal, hepatotoxin, icthyotoxic, immunosuppressive, molluscidal, neurotoxic, no activity, PBDu binding, tumor promoter, protein kinase activator, skin irritant, sunscreen pigment, toxin (**26**)
Nostocales (**41**)	126	Anticancer, antifungal, antimalarial, anti-HIV, cardioactive, hepatotoxic, antimicrobial, antimitotic, anti-inflammatory, antiviral, cytotoxic, enzyme inhibitor, toxin, neurotoxin, pigment, no activity (**16**)
Stigonematales (**6**)	16	Antifungal, antibiotic, anticancer, antimitotic, cytotoxic, herbicidal, no activity (**7**)

appreciated as early as 1500 BC, when *Nostoc* species were used to treat gout, fistula and several forms of cancer.[12] Yet, prior to the 1990s, limited investigations were undertaken on the isolation of biologically active natural products from cyanobacteria. During the 1990's, workers at several laboratories, including those of Richard Moore[9] (University of Hawaii), William Gerwick[13] (Oregon State University) and colleagues, had begun to screen extracts of cyanobacteria, mostly strains of *Microcystis* and *Anabaena* sp., for various biological activities, using predominantly mechanism- and enzyme-based assays.[14,15] Currently, published data indicates that these groups have screened over 4000 strains of freshwater and marine cyanobacteria. The very high incidence of novel, biologically active compounds isolated by these researchers to date (6% having anticancer (antiproliferative) activity)[15] indicates that cyanobacteria are a rich source of potentially useful natural products.

It is impossible to completely separate both freshwater and marine cyanobacteria, and focus solely on marine cyanobacteria, due to the fact that the same species may be able to grow within both environments and produce similar or different natural products.[9] As a result, this review reports on cyanobacteria that are present within the MarinLit reference database,[16] by definition concentrating on marine-derived natural products found within the literature.

2. Marine toxins

Freshwater cyanobacterial blooms that have been implicated in human and livestock intoxications have been extensively studied.[155,156] Bloom taxa, members of the Orders Nostocales and Chroococcales (Table 1), including *Anabaena*, *Aphanizomenon*, *Microcystis* and *Nodularia* species, exhibit severe neuro-, cyto- and hepato-toxicity to a variety of mammals (including humans), birds, livestock, fish and invertebrates (including zooplankton).[155,157] Blooms of marine cyanobacteria are also becoming an increasingly familiar occurrence within the tropical and sub-tropical regions of the world. Bloom taxa, members of the Order Chroococcales, Oscillatoriales (Table 1), including *Synechocystis*, *Hormothamnion*, *Oscillatoria* and *Lyngbya* species, grow along shallow, sheltered, back reef zones and periodically develop into dense blooms that often wash ashore and accumulate *en masse*.[158,159]

Only a few reports of toxicity amongst true marine cyanobacteria have appeared in the literature. Unlike the freshwater species, the marine cyanobacteria have not presented serious health and economic problems. Possibly the only exceptions are *Lyngbya majuscula*, which is responsible for sporadic outbreaks of a contact dermatitis known as 'swimmers itch',[160] *Hormothamnion enteromorphoides*, a tropical marine cyanobacterium which produces the antimicrobial and cytotoxic, lipopeptide hormothamnin A **1**[114,161] and *Synechocystis* sp. which produces the cytotoxic cyclic C_{11} metabolites nakienone A **2** and B **3**, found overgrowing coral (*Acropora* sp.) in Okinawa.[33]

Many of the cyanobacterial species, however, which are classified as freshwater cyanobacteria, have also been isolated from marine sources (Table 1). This is either as a direct result of run-off and outflow from river ways and estuaries, or due to the fact that these species are able to survive and indeed flourish within the marine environment. Many species of cyanobacteria can adjust to a diversity of growth conditions and habitats, albeit often producing different toxic principles to those observed from freshwater species. As can be seen in Table 1, there are three Cyanophyte orders that produce the vast majority of toxic compounds, these being Chroococcales, Oscillatoriales and Nostocales. Table 1 (summary table), demonstrates quite succinctly that over 40 different Nostocales species, the majority of which are *Anabaena* and *Nostoc* sp. produce over 120 natural products, these almost entirely being secondary metabolites, which can be classified as biotoxins. The associated activities, such as anticancer, antifungal, antimalarial, anti-HIV and antimicrobial, are a direct result of their cytotoxic activity. In a related study, cytotoxic antibiotics discovered during the study of Patterson et al.[7] included acutiphycins, indolcarbazoles, mirabilene isonitriles, paracyclophanes, scytophycins, tantazoles, tolytoxin, toyocamycin and tubercidin. The main orders of cyanobacteria found to produce these compounds were Nostocales and the Stigonematales.

The toxins of cyanobacteria constitute a major source of natural product toxins that are found in surface supplies of both freshwater and seawater. Species and strains from all of the common planktonic cyanobacterial genera including *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Microcystis*, *Nodularia*, *Nostoc*, *Oscillatoria*, *Lyngbya*, *Scytonema* and *Tolypothrix* produce biological toxins (Table 1). Other genera including *Coelosphaerium*,

Fisherella, *Gloeotrichia*, *Gomphosphaeria*, *Hapalosiphon*, *Schizothrix*, *Spirulina*, *Symploca* and *Trichodesmium* have also been found to be toxic but, as yet, no toxin has been isolated and characterized from these genera.[162,163]

Several systematic surveys within Europe and the United States have concluded that the two most commonly isolated groups of cyanotoxins are the alkaloid neurotoxins and the cyclic peptide hepatotoxins.[164–167] Neurotoxins are produced by species and strains of *Anabaena*,[95] *Aphanizomenon*,[168] *Oscillatoria*[162] and *Trichodesmium*.[169] These genera are now known to be the source of five chemically defined neurotoxins. These neurotoxins include anatoxin-a **4**, the first toxin to be chemically and functionally defined, isolated from *Anabaena flos-aquae*, *Aphanizomenon flos-aquae* and *Oscillatoria* sp.,[162,170] and which acts as a potent post-synaptic cholinergic nicotinic agonist. Anatoxin-a is not the only neurotoxin to be produced by *Anabaena* sp., since anatoxin-a (s) **5** [(s)—salivation, noted in laboratory mice injected with toxin] was also found to be produced by *Anabaena flos-aquae*,[103,104] whilst, saxitoxin dihydrochloride **6** has been isolated from *Aphanizomenon flos-aquae* and *Anabaena circinalis*, and together with the analogue, neosaxitoxin, constitutes the major toxin responsible for red tide paralytic shellfish poisoning (PSP).

Hepatotoxins are the most commonly encountered toxins involving cyanobacteria, and include the cyclic peptides, microcystin **7** and nodularin **8**.[155] *Microcystis aeruginosa* and *Nodularia spumigena* are among the many forms that synthesize toxins destructive to liver cells. These two species produce the seven amino acid peptide, microcystin, and the five amino acid peptide, nodularin, respectively. To date, over 50 different variants of microcystins have been isolated (**7** lists the most commonly isolated) from species and strains of *Anabaena*, *Hapalasiphon*, *Microcystis*, *Nostoc* and *Oscillatoria*. The revelation that cyanobacterial hepatotoxins can inhibit protein phosphatases has raised the disturbing possibility that human exposure to nonlethal doses might contribute to the development of cancer. Apparently, these toxins do not seem to initiate a cell's progression towards becoming cancerous, but, once another factor has triggered early changes, the hepatotoxins promote development of further carcinogenic alterations.[171,172] Yet, as is true of the neurotoxins, the effects of the hepatotoxins are not all detrimental. Since they affect the cytoskeleton, they are now being used as tools to probe the workings of this cellular scaffolding. Additionally, because microcystins impede protein phosphatases, they are aiding investigators in the effort to understand the mechanism of action of these enzymes.

One of the most toxic genera of cyanobacteria belonging to the order Oscillatoriales is *Lyngbya*, which are filamentous cyanobacteria abundant within tropical and sub-tropical waters. These are responsible for such cytotoxic compounds as antillatoxin **9**, aplysiatoxin **10**, debromoaplysiatoxin **11** and lyngbyatoxin A **12**, B and C. Oscillatoriales species produce a vast number of secondary metabolites, the current count being 197 compounds from only 15 species (Table 1 (summary table)), which translates into an average of 13 compounds isolated from each species. As can be seen in Table 1, however, the major Oscillatoriales species that produces secondary compounds is *L. majuscula*. Once this organism is taken into account, the results collate with those obtained from Nostocales and Chroococcales species at approximately 3–5 compounds from each 'bioactive' cyanobacterial species.

So, for what reason are all these chemicals made? Interestingly, in general, only those organisms which lack an immune system are prolific producers of secondary metabolites.[173] It is likely that cyanobacteria synthesize poisons to ward off attack by other planktonic species. Carmichael et al.[174] have found that cyanobacterial neurotoxins and hepatotoxins can be extremely harmful to the zooplankton living in the marine environment. The toxins may therefore be directly lethal (especially the neurotoxins), or they may reduce the number and size of offspring produced by the creatures that feed on cyanobacteria. DeMott et al.[175] have also found that zooplankton species generally do not eat cyanobacteria capable of producing toxins, unless there is no other food available, when they often attempt to modulate the amount they take in to ensure that they avoid lethal dosages.

3. Marine bioproducts

Until recently, toxic principles dominated the spectrum of biological activities isolated, although ecology would suggest that antimicrobial and antiviral agents are the most likely compounds that might be isolated from cyanobacteria.[176] This may partly be due to the application of cytotoxicity-directed screening assays, which constitute part of several of the international cancer institute's extensive natural product discovery programmes. Fig. 1, modified from Jaspars et al.[177] shows the analysis of published data up to 1996 (208 compounds). It has to be considered, however that defence strategies are necessary to survive in the highly competitive marine environment, thus resulting in a tremendous diversity of highly toxic compounds affecting numerous targets involved in eukaryotic cell signalling processes. Following the current decline in many natural product discovery programmes globally,[178] research has focused on adapting current cyanobacterial collections and cyanobacterial-derived compounds for screening in new pharmaceutical and industrial assays. Fig. 2 shows the analysis of published data up to 2001 (550 references/424 compounds).[16] As a result, the diversity of

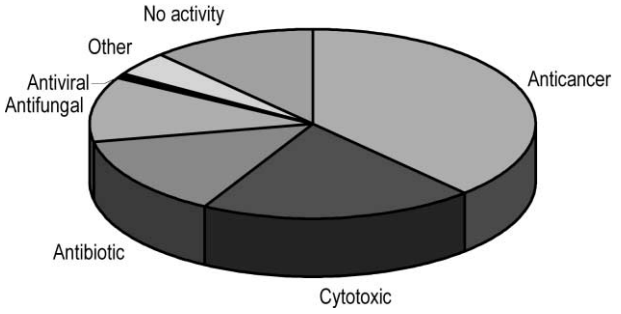


Figure 1. Reported biological activity of cyanobacterial compounds—1996 (208 compounds).[64]

1	Hormothamnin A	**8**	Nodularin
2	Nakienone A	**9**	Antillatoxin
3	Nakienone B	**10**	Aplysiatoxin
4	Anatoxin-a	**11**	Debromoaplysiatoxin
5	Anatoxin-a (s)	**12**	Lyngbyatoxin A
6	Saxitoxin dihydrochloride	**13**	Borophycin
7	Microcystin		

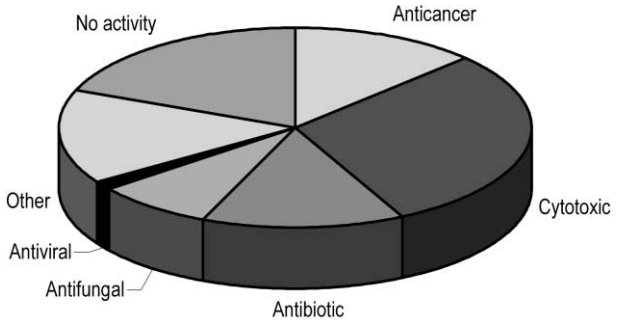


Figure 2. Reported biological activity of marine cyanobacterial compounds—2001 (424 compounds).[64]

biochemically active compounds has drastically increased to include enzyme inhibitors, herbicides, antimycotics, antifeedants, multi-drug resistance reversers and anti-malarial and immunosuppressive agents.[64,67,154,179,180] This can be demonstrated by the large increase (from 4 to 15%) of cyanobacterial compounds with 'other' activity from 1996 to 2000.

4. Exciting discoveries from marine cyanobacteria

Although terrestrial cyanobacteria are a well recognized source of biologically active, structurally unique natural products,[7,8,15] marine species have received considerably less attention.[9] Perhaps the most significant recent results have been the discoveries of borophycin **13**, cryptophycin 1 **14** and 8 **15** and cyanovirin **16**. Borophycin **13** is a boron-containing metabolite isolated from marine strains of the cyanobacteria *Nostoc linckia* and *Nostoc spongiaeforme* var. *tenue*.[6,181] Borophycin, which is related both to the boron-containing boromycins isolated from a terrestrial strain of *Streptomyces antibioticus* and to the aplasmomycins isolated from a marine strain of *Streptomyces griseus* (actinomycetes), exhibits potent cytotoxicity against human epidermoid carcinoma (LoVo) and human colorectal adenocarcinoma (KB) cell lines,[6] and has recently been found to exhibit antimicrobial activity.[181]

Cryptophycin 1 **14** was first isolated from *Nostoc* sp. ATCC 53789 by researchers at Merck, and found to be a potent fungicide. It was, however, found to be too toxic, and disregarded as a natural product lead. Subsequently, Moore et al.[182] isolated this same compound from *Nostoc* sp. GSV 224, which exhibited potent cytotoxicity against human tumor cell lines and good activity against a broad spectrum of drug-sensitive and drug-resistant murine and human solid tumors. Nevertheless, **14** again appeared to be too toxic to become a clinical candidate. This led to a detailed structure–function study by Moore in collaboration with Carmichael,[182] and resulted in the isolation of cryptophycin 8 **15**, a semisynthetic analogue which proved to have a greater therapeutic efficiency and lower toxicity than **14** in vivo. Although neither cryptophycin, nor any of its analogues (the current number being 52),[183] have entered clinical trials to date, interest in these compounds continues. Recently, Eli Lilly and Corporation (Indianapolis, IN, USA) (US Patent No. 6,020,512) have shown interest in developing a novel method for the production of cryptophycin intermediates, presumably for further study. In any case, the work of Moore and Carmichael[182] (US Patent Nos. 6,013,626, 5,955,423 and 5,952,298) shows quite eloquently that secondary metabolites in their natural form will not necessarily lead directly to commercialization, but the activity induced by these natural compounds may be exploited in structure–function studies to develop analogues with greater and more focused activities.

Cyanovirin (CV-N, cyanovirin-N) **16**, a 101 amino acid protein, has recently been placed on an accelerated track for clinical development for use as a virucidal agent.[127] The viron-receptor binding activity of cyanovirin was first discovered in 1997, from an extract of *Nostoc ellipsosporum* (F90783, Q68D170) isolated in the early 1980s. The USA's National Cancer Institute (NCI) discovered that cyanovirin is a fusion inhibitor of HIV, somehow preventing the surface receptor of HIV from undergoing the conformational changes that enable the viral and cell membranes to fuse, thus allowing the transfer of the viral genetic material into the host. CV-N was found to have potent activity against all immunodeficiency viruses (HIV-1, M- and T-tropic strains of HIV-1, HIV-2, SIV (simian) and FIV (feline)).[127] Recently, several patents have been lodged (US Patent Nos. 6,015,876, 5,998,587, 5,962,653, 5,843,882 and 5,821,081) to protect this new method of HIV prevention. Initial observations suggest that CV-N proteins are not present in other species of cyanobacteria, that there are no known open reading frame homologues and that the normal biological function is unknown. A systematic search for such analogues has not, however, been conducted, and it would seem highly improbable that such a protein would have evolved in only one of the 100 or more species of the genus *Nostoc*, or indeed within related species. The use of CV-N and any analogues, if discovered, in structure–function experiments could lead to an entirely new class of anti-HIV drug being developed.

5. Marine chemical ecology

Although there is an ever-growing compilation of research regarding the chemistry and biochemistry of marine toxins and potential drug leads, as discussed in Sections 3 and 4, little experimental evidence exists to establish the full ecological significance of most cyanobacterial metabolites.[158,184,185] The authors believe that a better understanding of ecological relationships and interactions which are present, in particular marine niches where the target micro-organisms are found, allows the mechanistic design of artificial media which more closely resemble that micro niche and thus leads to a much greater percentage culturability. In addition, secondary metabolite production is often very sensitive to environmental factors. Substantial time and effort must, therefore, be expended to determine the chemical and physical conditions appropriate for optimum growth and drug production. Even when seemingly stable strains of drug-producing cyanophytes are obtained using conventional techniques, drug production sometimes disappears on repeated subculturing.[9] The authors therefore recognize the invaluable research that the small band of chemical ecologists produce annually. Current research shows that compounds produced by marine

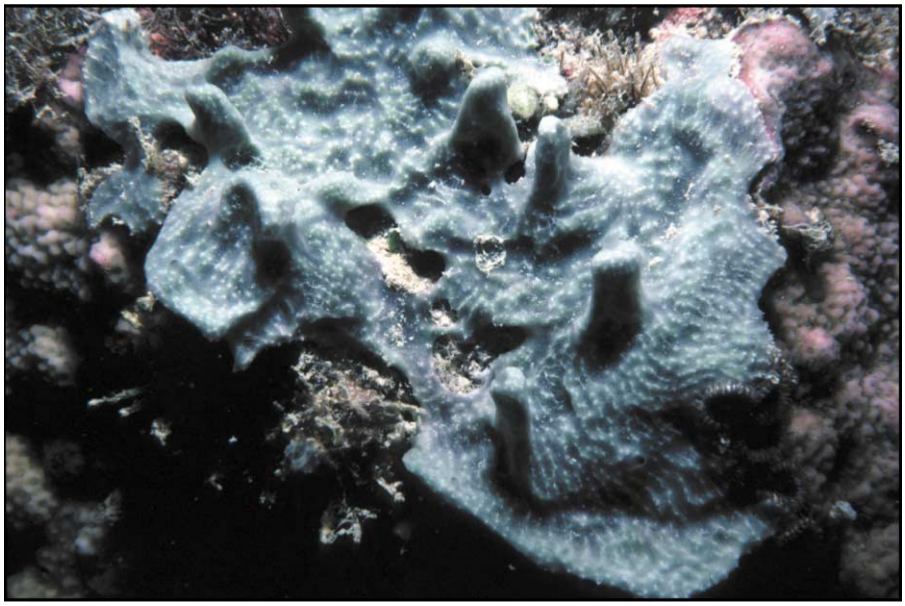


Figure 3. Close-up photo of *Dysidea herbacia*, taken on the Great Barrier Reef, Australia. Source: Australian Institute of Marine Science Photo Collection.

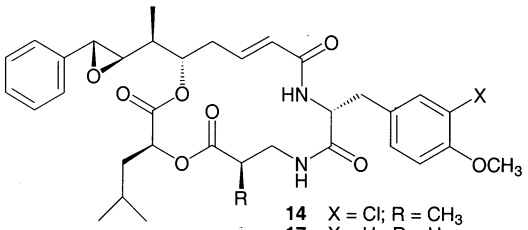
cyanobacteria deter feeding, that cyanobacteria participate in symbiotic relationships with several marine invertebrates and that many are responsible for extremely active compounds previously attributed to marine invertebrates. Several case studies are presented below.

It is fascinating that many marine natural products found in sponges and their predators closely resemble cyanobacterial secondary metabolites. Recently, a cryptophycin-type cytotoxin, arenastatin A **17**, was isolated from the sponge, *Dysidea arenaria* by Kobayashi and Kitagawa,[186] and its relative and absolute stereochemistry unambiguously established by total synthesis. It is well known that secondary metabolites in some species of *Dysidea* are produced by symbiotic cyanobacteria,[45,187,188] but in this example it is not known if a cyanobacterial symbiont is responsible for the production of arenastatin A **17**, although this seems quite possible. In a second related example, the trichloromethyl portion of barbamide **18** closely resembles the trichloromethyl portion of dysidin **19**, a polychlorinated amino acid derivative found in the sponge, *Dysidea herbaceae* (Fig. 3).[189] Microscopic investigations of *Dysidea* have shown it to be rich in symbiotic filamentous cyanobacteria. A flow-cytometric separation of the symbiont, *Oscillatoria spongeliae*, from the sponge cells suggested that the polychlorinated amino acid derivatives were associated with the cyanobacterial filaments.[45,189] Orjala and Gerwick[66] found these structurally similar components, which they isolated from *L. majuscula*, thus providing further support for the cyanobacterial origin of these metabolites.

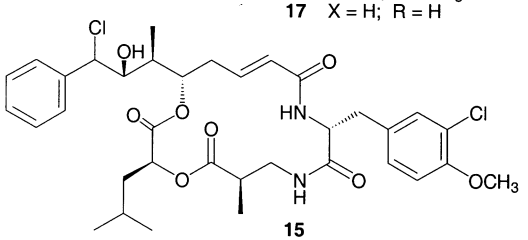
The most striking examples of structural similarity are a large number of cytotoxic compounds isolated by marine natural product chemists from sponges and molluscs that are scytophycin-like **20**–**24** (natural products isolated from *Scytonema mirabile* BY-8-1),[182] i.e. the swinholides **25**, ulapualides, kabiramides, halichondramides, misakinolides, mycalolides, sphinxolides and aplyronines. Like the scytophycins, many of these latter compounds appear to be cytotoxic because they disrupt microfilament organisation. With the exception of the aplyronines from the sea hare *Aplysia kurodai*, representatives from the other classes of compounds are highly toxic to animals and show only marginal antitumor activity. It is thought that these compounds probably accumulate in the mollusc through the diet, and may have a cyanobacterial origin.

Certain ascidians (tunicates) harbour cyanobacterial symbionts and produce antitumor compounds. Didemnin B **26**, for example, is a highly cytotoxic, cyclic lipopeptide from the Caribbean tunicate *Tridemnum solidum*, and was the first marine natural product to be evaluated in human clinical trials[39] for the treatment of various cancers (melanoma, lymphoma, astrocytoma, and carcinoma of the cervix, prostate, lung, colon, rectum, ovary and kidney).[190–195] Unfortunately, the results of the Phase II trials carried out over the past decade have been disappointing, with cardiotoxicity leading to its ultimate downfall.[37] Currently, an analogue of **26** is undergoing clinical investigation in Europe and the USA, with the early signs looking promising.[196–198] Whether the symbiotic cyanobacterium *Synechocystis trididemni*, isolated from this ascidian, is involved in the biosynthesis of the didemnins is still open to debate.

Some promising antitumor agents have also been isolated from sea hares that feed on cyanobacteria. The opisthobranch sea hares *Stylocheilus longicauda* and *S. striatus* (Fig. 4) are reef grazers that specialize on *L. majuscula* and sequester cyanobacterial metabolites, which may function in defence against predators.[199–201] Actually, the association of *Stylocheilus* with *Lyngbya* has a far greater importance for the mollusc than just food. Switzer-Dunlap and Hadfield have demonstrated that the veligers of *S. longicauda* undergo metamorphosis preferentially on the thalli of *L. majuscula*, using an unknown cyanobacterial metabolite as the hormone to induce this change,[202] while Nagle et al.[203] discovered that several compounds isolated



14 X = Cl; R = CH_3
17 X = H; R = H



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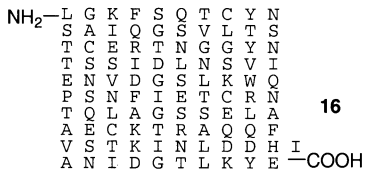

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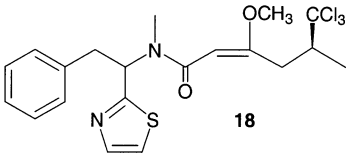

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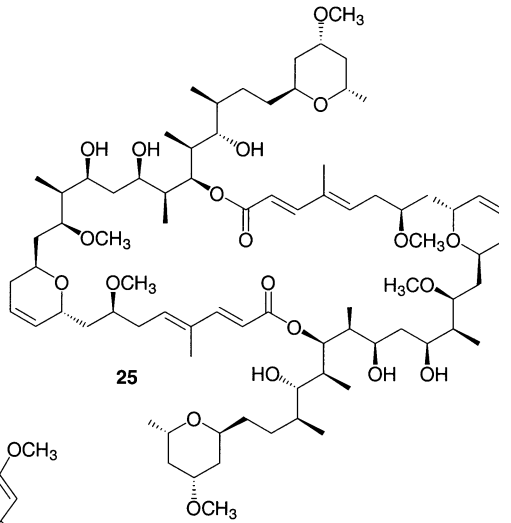

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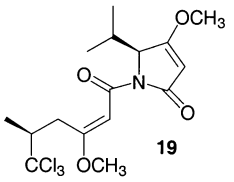

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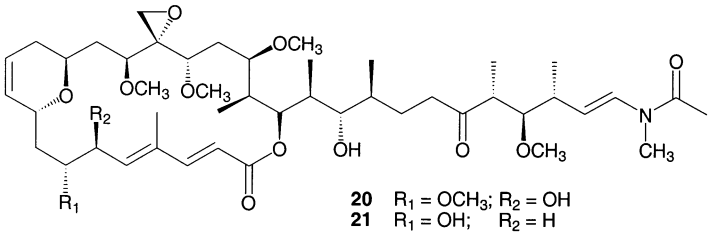
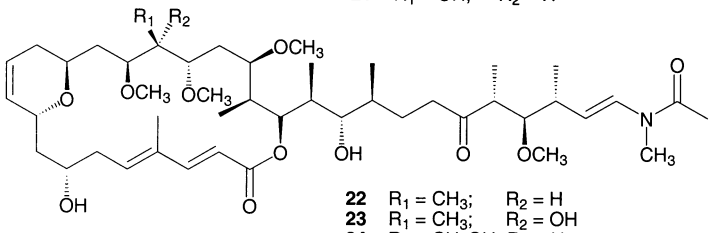
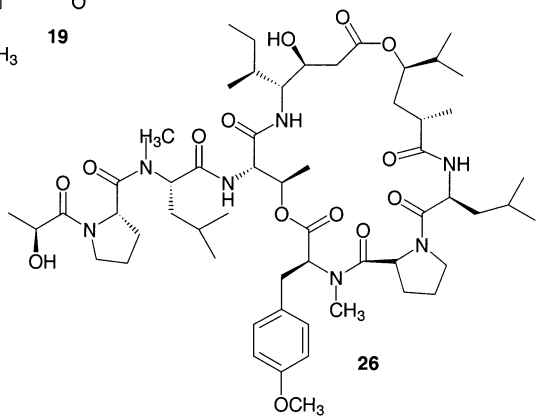


20 $R_1 = OCH_3$; $R_2 = OH$
21 $R_1 = OH$; $R_2 = H$



22 $R_1 = CH_3$; $R_2 = H$
23 $R_1 = CH_3$; $R_2 = OH$
24 $R_1 = CH_2OH$; $R_2 = H$



26

14	Cryptophycin 1	**21**	Scytophycin B
15	Cryptophycin 8	**22**	Scytophycin C
16	Cyanovirin	**23**	Scytophycin D
17	Arenastatin A	**24**	Scytophycin E
18	Barbamide	**25**	Swinholide H
19	Dysidin	**26**	Didemnin B
20	Tolytoxin B		

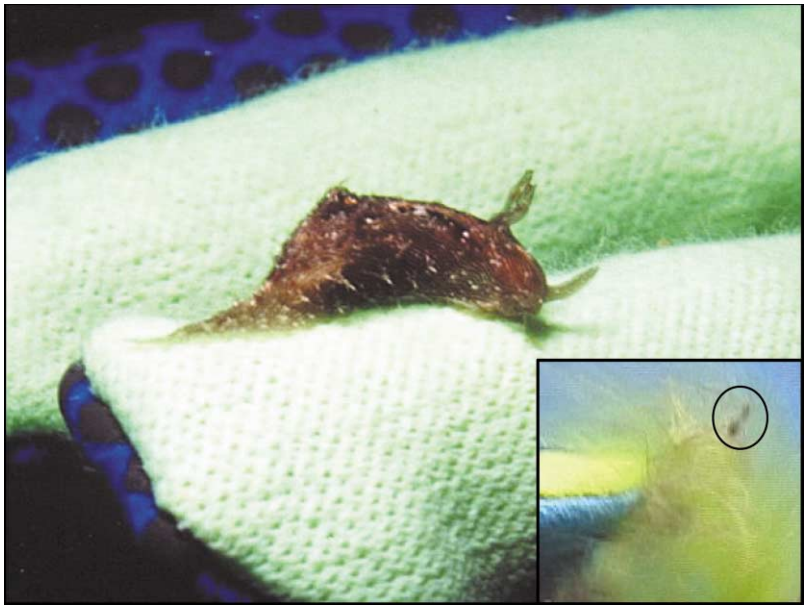


Figure 4. Close-up photo of *Stylocheilus striatus* taken in Hawaii, USA; this sea hare has been documented to graze on *Lyngbya majuscula* (June 2000). The insert shows *S. striatus* located on *L. majuscula*. Source: Courtesy of Ursula Keuper-Bennett (Turtle Conservation Group, Turtle Trax, USA).

from cyanobacteria deter feeding by *S. longicauda* at ecologically relevant concentrations. It has additionally been suggested that *Stylocheilus* dietary selection may be regulated by the concentration of specific chemical cues produced by these cyanobacterial species.[203]

Over the past decade, several biologically active compounds first isolated from *S. longicauda* have been ultimately tracked to cyanobacteria. The isolation of majusculamide C **27** from marine cyanobacteria, for example, provided the first indication that the structurally related dolastatins, first isolated from sea hares, may have been of dietary origin. Several dolastatins and dolastatin analogues have recently been isolated from the marine cyanobacterial species *Symploca hydnoides* (Kutzing and Gomont),[93] *L. majuscula* (Gomont)[56,62] and from mixed cyanobacterial assemblages.[52] Dolastatin and dolastatin analogues are potent cytotoxins, dolastatin 10 **28**, dolastatin 15 **29**, cermadotin and LU103793[204–206] currently being in clinical evaluation as potential new anticancer drugs. In particular, **28** and cermadotin have recently been recommended for Phase II clinical trials.[206–208] Although the origin of the cyanobacterial species which produces dolastatin 10 **28** has not been determined to date, several of the 'statin' family of compounds (dolastatin 3 **30**, dolastatin 11 **31**, dolastatin 12 **32**, dolastatin G, lyngbyastatin 1 **33**, lyngbyastatin 2 and several structural analogues (Table 2)) have been isolated from *L. majuscula* and *S. hydnoides*.[52,56,62] The presence of similar compounds in *L. majuscula* and *S. hydnoides*, suggests that the metabolic pathway for the production of **28** is at least present within these cyanobacteria, and may possibly be the source of this compound.

Over the past five years, several additional examples have been discovered which demonstrate that compounds which were originally thought to derive from marine invertebrates are actually cyanobacterial in origin. Many of these compounds have been isolated from the authors' target organism, *L. majuscula*, and include the α,β-epoxycyclohexenone moiety of malyngamide H **34**, grenadadiene **35**, debromogrenadadiene and grenadamide **36**,[70] which are structurally related to compounds previously isolated from the sea hare *S. longicauda*.[66] Interestingly, the Dhoya unit present within yanacamides A **37** and B **38** has previously been found only in kulolide-1 **39** and kulokainalide-1 **40** metabolites isolated from the marine mollusc *Phillinopsis speciosa*.[71] A study by Scheuer and coworkers[5,209] has shown that *P. speciosa* preys on the herbivorous sea hare *S. longicauda*. Thus, the discovery of the yanucamides **37** and **38** from a marine cyanobacterium substantiates the hypothesis that marine cyanobacteria are the probable source of the kulolides **39** and **40** and their related metabolites,[71] and shows quite distinctly that, through an understanding of the chemical ecology associated with cyanobacteria, a direct link back to a cyanobacterial source can be determined.

The production of deterrent metabolites may be vital to cyanobacterial survival in herbivore-rich coral reef environments. Production of deterrent metabolites may be vital to large-scale mat and bloom formation, especially in the presence of grazers. Recent studies suggest that fast-growing cyanobacteria that produce few secondary metabolites may associate with slower-growing chemically-rich species, such as *L. majuscula*, thus providing a measure of defence against herbivory.[210]

There is also at least one example where cyanobacteria form symbiotic or commensal relationships with other marine bacteria in order to overcome environmental stresses, via biofilm formation. In a study by Gil-Turnes,[211] it was observed that cyanobacteria also form highly specific, symbiotic relationships with other bacteria. As part of an investigation on the tropical, filamentous cyanobacterium *Microcoleus lyngbyaceus* (also known as *L. majuscula*)

Table 2. Compounds isolated from *Lyngbya majuscula* from various locations. Abbreviations used throughout are summarized at the end of this table. Included within Table 2 (highlighted box) is a summary of the total number of compounds and activities ascribed to *L. majuscula*

Compound	Activity	Screening	Comments	Reference
Antillatoxin[a] *epiantillatoxin*	Brine shrimp toxicity, icthyotoxic, neurotoxic	Brine shrimp and icthyotoxic activity	Neural necrosis through *N*-methyl-D-asparate receptor mechanisms	48,241,242
Aplysiatoxin *debromoaplysiatoxin anhydro-debromoaplysiatoxin*	Antiproliferative, anticancer, PBDu binding, tumor promoter	KB, leukemia cell line, phorbol dibutyrate (PDBu) receptor binding assay, NCI	Debromoaplysiatoxin and anhydro-debromoaplysiatoxin have undergone NCI testing	23,82,239,243–245
Apramide A–G	No activity	KB, LoVo, antibacterial, antifungal and protease-inhibitory activity	Structurally related to the carmabins and microcolins	58
Barbamide	Brine shrimp toxicity and molluscidal toxicity	Brine Shrimp and molluscidal assay	Barbamide producing strain in culture	66
γ-Butyrolactone +*-a(S)-butyramido-γ-butyrolactone, butyrolactone 3-γ*	No activity, cytotoxic	Brine shrimp assay, HCT-116 and KB		73
Carmabin A–B	Anticancer, antiproliferative (1991)/no activity (1993)	MRC-5, NCI	Cause of initial antiproliferative activity unknown	54
Curacin A–D	Anticancer, antimitotic, anti-inflammatory, antiproliferative, brine shrimp toxicity, immunosuppressive, herbicidal	Brine shrimp assay, L1210, KB, MRC-5, AB1, MCF-7, NCI, tubulin assembly and colchicine to tubulin binding	Antimitotic mode of action similar to a number of conventional antineoplastics, but structure is novel; entered Phase I NCI trials, currently sold as molecular probe. curacin A-producing strain in culture	50,59,66,74,241,246–248
(*S*)-(−)-3,4-Dihydroxybutanoic acid γ lactone	No activity	HL-60	Opposite configuration of lactone generated by hydrolysis of oscillatoxin A	239
N,7-Dimethylindole-3-carboxaldehyde	No activity	Brine shrimp assay, HCT-116 and KB		73
Dolastatin 3, G, 11, 12 *epidolastatin 12 homodolastatin 3*	Anti-HIV, anticancer, antiproliferative, brine shrimp toxicity	HIV-1, LoVo, KB, L1210, A-38, 17/Adr, CFU-GM, A-16/C, brine shrimp assay	Identical to majusculamide C except for an *N*-methylleucine residue substitution, inhibits the actin-dependent process of cytokinesis	52,56,62,63
Grenadadiene *debromogrenadiene*	Antiproliferative, anticancer	NCI	Bromide atom present within compound structure (unusual)	70
Grenadamide	Brine shrimp toxicity	Brine shrimp assay, cannabinoide receptor binding activity	Ichthyotoxicity through cannabinoid receptor binding activity	70
Hermitamide A–B	**A**: Icthyotoxic, **A** and **B**: brine shrimp toxicity and cytotoxic	Brine shrimp, icthyotoxic and molluscicidal assay, Neura-2a	First *Lyngbya majuscula* aromatized malyngamide-type compound	72
Kalkipyrone	Anticancer (modest), brine shrimp toxicity, icthyotoxic	Brine shrimp assay, goldfish assay, NCI	Novel γ-pyrone derivative isolated from assemblage with *Tolypothrix* sp.	51
Kalkitoxin	Brine shrimp toxicity, neurotoxic	Brine shrimp assay	Neural necrosis through *N*-methyl-D-asparate receptor mechanisms	48
Kororamide	No activity	HIV-1		62
Laxaphycin A–B	**A**: No activity **B**: cytotoxic, antifungal	Antifungal, CCRF-CEM, CEM/VLB$_{100}$, CEM/VM-1	Laxaphycin A potentialises the activity of Laxaphycin B	227,249
Lyngbyabellin A	Anticancer, cytoskeleton disruption	KB, LoVo, A-38, A-16/C, A-10, cytoskeleton disruption, topoisomerase inhibition	Microfilament specific disruption of cytoskeleton; same compound (dolabellin) found in sea hare	57
Lyngbyacarbonate	No activity	Brine shrimp assay, HCT-116 and KB	New class of secondary metabolite	73

Table 2. (*continued*)

Compound	Activity	Screening	Comments	Reference
Lyngbyastatin 1–2 *epilyngbyastatin 1* *norlyngbyastatin 2*	Anticancer, antiproliferative, brine shrimp toxicity	Brine shrimp assay, L_oV_o, KB, L1210, A-38, 17/Adr, CFU-GM, A-16/C, human solid tumor assay, Corbett assay	Analogue of dolastatins 11 and 12 inhibits the actin-dependent process of cytokinesis	52,56
Lyngbyatoxin A–C	Cytotoxic, icthyotoxic, tumor promoter, protein kinase C activator, skin irritant	Mouse toxicity assay (intraperitoneal), baitfish toxicity assay, P-388, rabbit aorta contractions, HL-60, two-stage mouse skin carcinogenesis study	Gross structure closely related to that of teleocidin A-1, a toxic substance found in the mycelia of *Streptomyces* sp.	216,250–252
Majusculamide A–D *5,7-Normajusculamide C*, *deoxymajusculamide D*	Antifungal, antimycotic activity, cytotoxic	Antifungal and antimycotic activity against (*Saccharomyces pastorianus*), CCRF-CEM	**D**: Absolute stereochemistry not determined due to impurities	63,216,220,253
Malyngamide A–U *deoxymalyngamide C* *malyngamide D acetate* *malyngamide I acetate* *Isomalyngamide A–B*	Antifeedant, antimicrobial, brine shrimp toxicity, cytotoxic, immunosuppressive, icthyotoxic	Antimicrobial activity, KB, immunosuppressive cell line assay, goldfish, brine shrimp and molluscicidal assay, NCI, Neura-2a	**H**: First non-chlorine-containing amide of 7-methoxytetradec-4(*E*)-enoic acid; **J**: Selected for in vivo testing due to unusual profile in NCI assays; **Q** and **R**: first with altered geometry about C-6	47,60,61,66,216–219
Serinol-derived malyngamide 1–2	Anti-HIV (modest)	Anti-HIV assay	Identified as *Lyngbya* sp., most probably *Lyngbya majuscula*	254
(±) Malyngolide	Antibiotic	Antibiotic, antimicrobial and antifungal activity		69,255,256
(±) 7-Methoxytetradec-4(*E*)-enoic acid	Anitmicrobial, immunosuppressive	Antimicrobial assay, KB, immunosuppressive cell line assay	Intermediate in the synthesis of malyngamides (shallow water)	70,72,217,254,257
7-Methoxy-9-methylhexadec-4(*E*)-enoic acid	Anitmicrobial, immunosuppressive	Antimicrobial assay, KB, immunosuppressive cell line assay	Intermediate in the synthesis of malyngamides (deep water)	70,72,217,254,257
Microcolin A–C	Anticancer, antiproliferative, cytotoxic, immunosuppressive	A 549, P-388, immunosuppressive cell line and antiproliferative assays	Microcolin A and B targeted for further study (more potent than cyclosporin A)	158,258
Monoterpene	No activity	Brine shrimp toxicity, HCT-116 and KB assay	Possible subunit of lyngbyatoxin	73
Oscillotoxin A	Brine shrimp toxicity, tumor promoter	Brine shrimp assay		62,239
Pitiamide A–B	Antifeedant	Antifeedant assay	Relative instability of this compound brings into question the nature of the true metabolite	65
Pukelemide A–G	No activity	Mouse toxicity (sub-cutaneous injection)		49,68
4,8-Dimethyl-6-*O*-(2′,4′-di-*O*-methyl-β-D-xylopyranosyl)-hydroxyquinoline	Icthyotoxic	Icthyotoxic, molluscidial activity assays	First report of hydroxyquinoline alkaloids from marine cyanobacterium	67
4,8-Dimethyl-6-hydroxyquinoline	No activity	Icthyotoxic, molluscidial activity assays	Unsure whether this is a true metabolite or formed by glycoside hydrolysis	67
Tanikolide	Antifungal, brine shrimp and molluscidal toxicity	Brine shrimp assay, icthyotoxic, molluscidial, antifungal and antimicrobial activity	Structurally related to malyngolide	69
Teleocidin A-1 and A-2, B *dihydroteleocidin B*	Tumor promoter, cytotoxic			259

Table 2. (*continued*)

Compound	Activity	Screening	Comments	Reference
Tumonoic acid A–C *methyl tumonoate A–B*	No activity	Brine shrimp assay	Possible role as antifeedant is currently being investigated (methyl esters possibly artifact)	53
Ypaoamide	Antifeedant	Antifeedant assay	Ypaoamide-producing strain in culture	64
Yanucamide A–B	Brine shrimp toxicity	Brine shrimp assay		71
109 compounds	*Activities*: Antimicrobial, antibiotic, anticancer, antifeedant, antifungal, anti-HIV, anti-inflammatory, antimicrobial, antimitotic, antimycotic activity, antiproliferative, brine shrimp toxicity, cytoskeleton disruption, cytotoxic, herbicidal, icthyotoxic, immunosuppressive, molluscicidal toxicity neurotoxic, no activity, PBDu binding, protein kinase C activator, skin irritant, tumuor promoter (**24**)			

Abbreviations: *17/Adr*, Mammary 17/Adr (Murine solid tumor) cell line; *CFU-GM*, Hematopoietic CFU-GM (normal) cell line; *A-10*, Fibroblast smooth muscle (normal) cell line; *HCT-116*, Human solid tumor (colon) cell line; *A-16/C*, Mammary adenocarconoma #16/C mouse assay; *HIV-1*, HIV-1 integrase activity in terminal cleavage and strand-transfer assays; *A-38*, Colon adenocarcinoma 38 (murine solid tumor) mouse assay; *HL-60*, Human promyelocytic leukemia cell line; *AB1*, Chinese hamster tumor cells; *KB*, Human nasopharyngeal carcinoma cell line; *A 549*, Anti tumor assay; *L-1210*, Murine leukemia cell line; *CA46*, Burkitt lymphoma cell line; *LoVo*, Human colon adenocarcinoma cell line; *CCRF-CEM*, Parent drug sensitive human leukemic lymphoblasts; *MCF-7*, Breast cancer cell line; *CEM/VLB100*, Vinblastine-resistant subline which presents an MDR phenotype; *MRC-5*, Human embryonic lung cell line; *CEM/VM-1*, a typical multidrug resistance (MDR) cell line; *Neura-2a*, Neuroblastoma cell line; P-388, Lymphocytic mouse leukemia cell assay; *NCI*, 60-cell line bioassay. The screening procedure employed a diverse, disease-oriented panel consisting of 60 different human tumor cell lines organized into seven disease-specific sub-panels. The extract was tested over a wide range of concentrations for cytotoxic or growth-inhibitory effects against each cell line comprising the panel. The seven sub-panels represented diverse histologies (leukemias, melanomas, and tumors of the lung, colon, kidney, ovary and brain).[10]

[a] Italicised compounds are known analogues of the major compounds described and exhibit the same activities, unless otherwise stated.

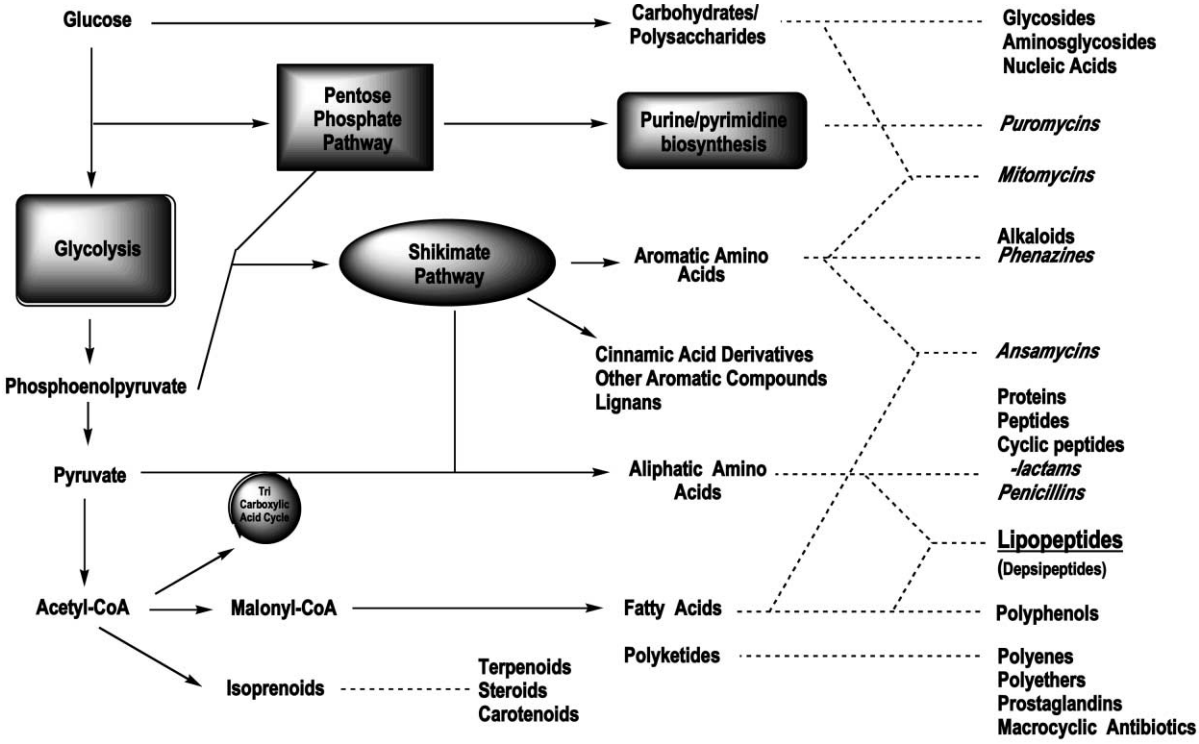


Figure 5. Core pathways to microbial secondary metabolites [modified from Grabley et al.[258]] including the known metabolic pathways for several antibiotics produced by actinomycetes and the postulated pathway for lipopeptide production.

from Puerto Rico, the surface-derived bacteria from 65 sampling sites around the island were studied and compared. From this work, it was found that four specific strains of highly coloured bacteria were obtained only from the surface of this cyanobacterium. These strains were isolated neither from adjacent seawater samples nor from morphologically similar filamentous cyanobacterial species. All four strains of bacteria were found to produce the same antibiotic material, identified as a quinone.[211] This molecule possesses significant antifungal and antibacterial activities, and is likely to act as an antifoulant. Subsequently, Orjala et al.[67] isolated two minor hydroxyquinoline compounds **41** and **42** (Table 2) from a Curaçao strain of *L. majuscula*, which is claimed as the first report from a marine cyanobacterium. As shown above, however this same species of cyanobacteria has been shown previously to be associated with bacteria that produce quinone compounds from a geographically similar region. It is therefore speculated here that the two minor compounds discovered by Orjala et al. may be more correctly assigned to bacteria that live in a symbiotic or commensal relationship with *L. majuscula*. An important point to note is that due to the debate over correct nomenclature within cyanobacteria, these organisms have both a botanical and bacteriological name. That *M. lyngbyaceus* and *L. majuscula* are one and the same, seems to have been overlooked in this case.

6. Secondary metabolic pathways

Over the last 50 years, microbial products (e.g. pigments, alkaloids, toxins, antibiotics, carotenoids, lipopeptides, etc.) that serve no obvious function in the life of the organisms that produce them (known as secondary metabolites), have been isolated and their structures elucidated. In the marine microbial world, cyanobacteria are especially prolific producers of such compounds, many of which show biological activities, such as antibiotic, anticancer, anti-HIV and toxic activities.

While secondary metabolism has a rather restricted distribution, primary metabolism is universal among microbes. The end products of primary metabolism are energy and intermediates for synthesis of essential macromolecules such as lipids, proteins and nucleic acids. In well-regulated, metabolically efficient, wild-type microbes such as *Escherichia coli*, intermediates and end products of primary pathways do not accumulate, and they do not possess the genetic apparatus to make secondary metabolites. On the other hand, certain wild-type organisms possess genes for producing secondary metabolites in addition to genes necessary for growth. In such organisms, certain steps of primary metabolism lack regulation. This results in the over synthesis of intermediates and end products of primary pathways. Under metabolic stress, these accumulated pools of intermediates may induce subsidiary pathways to form secondary metabolites.

In the last two decades, with the emergence of molecular biology tools, particularly where applied to a group of Gram-positive bacteria belonging to the family Actinomycetales, entire sets or clusters of biosynthetic pathway genes have been cloned and examined at the molecular genetic level.[212] The results from these molecular genetic approaches have further clarified the pathways by which complex secondary metabolites are assembled, and have provided an opportunity to engineer new biosynthetic pathways that generate novel bioactive secondary metabolites,[213]

and to increase biochemical diversity for the discovery of new drug leads.[214,215]

The biochemical and molecular genetic analysis of the biosynthesis of numerous secondary metabolites has revealed that a limited number of core biosynthetic pathways are responsible for the formation of the majority of compounds described here, including lipopeptides (Fig. 5). The structural diversity of natural products generated by microorganisms appears to be predominantly a result of modifications and combinations of reactions from primary metabolic pathways. For example, variations in the pathways responsible for the combination of fatty acid biosynthetic machinery with acetyl-CoA biosynthetic pathways result in the formation of lipopeptides. Only occasionally completely novel reactions are found in secondary metabolism that are not precedent in primary metabolism. This may reflect the process by which secondary metabolic pathways evolve.

Through the process of natural selection, this new structure will be 'evaluated' to establish whether it confers a selective evolutionary advantage on the producing cells. If production of the compound is advantageous, this new biosynthetic capability will be 'adopted'. This process has been noted several times within *L. majuscula*, most notably within the structurally novel metabolite ypaoamide **43**.[64] Structural similarities between this compound and the malyngamides **44**–**61**,[47,60,61,66,216–219] pukeleimides **62**–**68**,[49,68] majusculamide D **69**,[220] and microcolin A **70**, B **71** and C,[158] suggests that common biosynthetic pathways may be employed by different chemotypes of *L. majuscula*. While methylated valine-derived *t*-butyl amino acids are relatively common amongst marine natural products, the unusual *t*-butyl lipid side chain of ypaoamide **43** has little biosynthetic precedent other than in antillatoxin **9**, an ichthyotoxic cyclic lipopeptide from a Curaçao strain of *L. majuscula*.[221]

Although research on the natural products chemistry of cyanobacteria is very active and has been recently reviewed,[15,182] biosynthetic studies have been few, especially with the marine strains. Anatoxin-a (s) **5**, is a potent neurotoxin produced primarily by *Anabaena flos-aquae*. Feeding experiments with stable and radiolabelled precursors established that all carbons of the triaminopropane backbone and the guanidine unit **5** are derived from L-arginine and that the three methyl carbons arise from L-methionine or other donors to the tetrahydrofolate C_1 pool. The cyclic lipopeptides microcystin-LR **7** has also been studied in great detail. The Adda unit was found to be synthesized by the polyketide pathway involving a putative phenylacteyl-CoA starter unit and four malonyl-CoA extensions. Sodium [1,2-$^{13}C_2$]acetate was incorporated at C1 through C8 and the remaining Adda backbone carbons were derived from L-[U-^{13}C]phenylalanine. L-[*Methyl*-^{13}C]-methionine labelled the 2-, 6- and 8-methyl and 9-methoxy carbons.[121] Conversely, all the Adda side chain methyl groups in the related cyclic lipopeptides nodularin **8** were shown to be derived from methionine.[38] Gerwick and coworkers have recently probed the origin of the trichloromethyl group in the molluscicidal *L. majuscula*-derived metabolite barbamide **18**.[222] Using feeding experiments with differently ^{13}C-labelled L-Leucines, chlorination was shown to occur exclusively at the *pro-S* methyl group of leucine, which is not activated by a double bond, as incorporation experiments with L-[U-^{2}H]leucine showed that leucine hydrogens were retained at C1, C2 and C3 in **18**.[222]

7. Bioprocess intensification

Recent estimates indicate that, of the newly-approved drugs reported to date, those of natural origin (classified as original natural products, products derived semi-synthetically from natural products or synthetic products based on natural product models) predominate.[3] In some cases, demand can be met by the total synthesis of the active metabolite, but, in many cases, this is not a viable option as synthesis may involve many steps, may be relatively expensive and may produce low overall yields due to poor selectivity. The advantage of microorganisms which can be cultured, such as cyanobacteria, is that a sustainable supply of a 'targeted' metabolite can be achieved. This phenomenon is coupled with the fact that many of the products derived from a macroorganism source, on further investigation have actually been derived from a microbial, usually cyanobacterial source, as shown in the previous sections.[4,5] The interest in marine microbes as a source of natural products is therefore both warranted and opportune. Although there has been some interest in the exploitation of cultured cyanobacteria to develop pharmaceutical compounds, the authors believe that the study of these secondary metabolites and their controlled long-term production is still in its infancy. Of the limited research, which has been undertaken, there has been little concerted effort to move the biotechnological process forward beyond the characterisation phase. Although a number of researchers have touched on aspects dealing with bioprocess intensification [see Refs. 223,224] (improvement in process efficiency and effectiveness), no programme has tried to increase product yield of cultured cyanobacteria via culture and reactor configuration manipulation, through to the commercialization stage. It is hoped that common fermentation principles can be developed for bacteria isolated from similar ecological niches, leading to the design of an intensification process. The authors believe the application of bioprocess intensification methods to the production of cyanobacterial natural products is likely to become an important strategy for improving supply of natural products,[225–227] and will address this topic in a subsequent review.

8. Lipopeptides

An analysis of the literature on natural products derived from marine cyanobacteria[16] shows that approximately 68% contain nitrogen (i.e. probably derived from amino acid metabolism). The natural products of many marine cyanobacteria contain an amino-acid derived fragment linked to a fatty acid-derived portion, forming compounds known as lipopeptides **72**. Further analysis of the 424 marine cyanobacterial natural products contained within the MarinLit database shows that 40.2% are lipopeptides (cyclic or linear) [amino-acid derived compounds], 5.6% are of pure amino acid composition, 4.2% fatty acids, 4.2% macrolides and 9.4% are amides (Fig. 6).

	R_1	R_2	R_3	
27	OCH_3	H	$CH_3CH_2(CH_3)CH$-	S at C-15
31	OCH_3	H	$(CH_3)_2CHCH_2$-	S at C-15
32	H	CH_3	$(CH_3)_2CHCH_2$-	epimeric at C-15
33	OCH_3	CH_3	$(CH_3)_2CHCH_2$-	epimeric at C-15

28

29

34

35

36

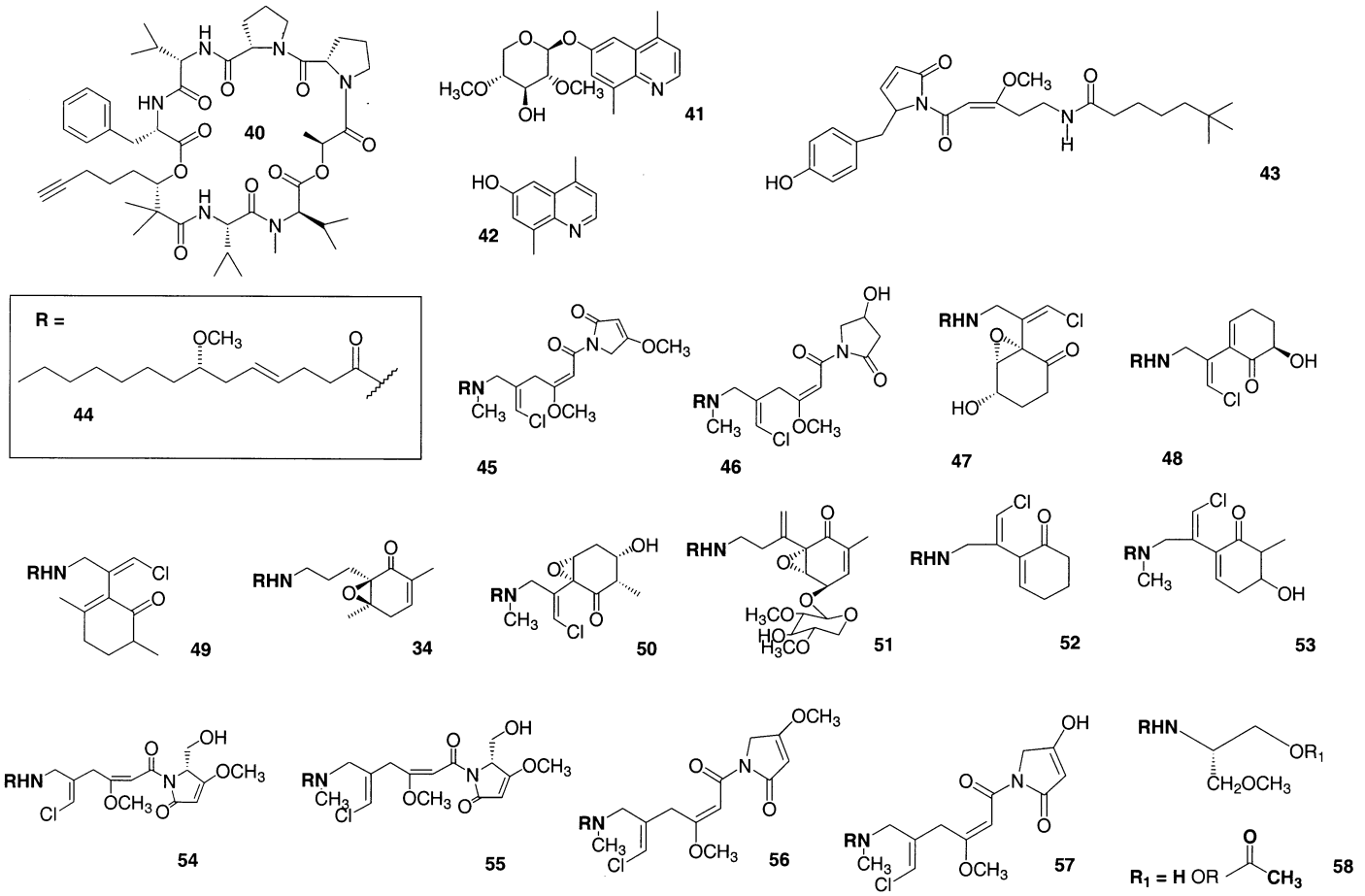
30

37

38

39

27	Majusculamide C	**34**	Malyngamide H
28	Dolastatin 10	**35**	Grenadadiene
29	Dolastatin 15	**36**	Grenadamide
30	Dolastatin 3	**37**	Yanucamide A
31	Dolastatin 11	**38**	Yanucamide B
32	Dolastatin 12	**39**	Kulolide 1
33	Lyngbyastatin 1		



40	Kulokainalide 1
41	Quinoline Alkaloid 1
42	Quinoline Alkaloid 2
43	Ypaoamide
44	(-)-7(*S*)-methoxytetradec-4(*E*)-enoic acid
45	Malyngamide A
46	Malyngamide B
47	Malyngamide C
48	Malyngamide F
49	Malyngamide G
50	Malyngamide I
51	Malyngamide J
52	Malyngamide K
53	Malyngamide L
54	Malyngamide Q
55	Malyngamide R
56	Isomalyngamide A
57	Isomalyngamide B
58	Seriol-derived Malyngamides

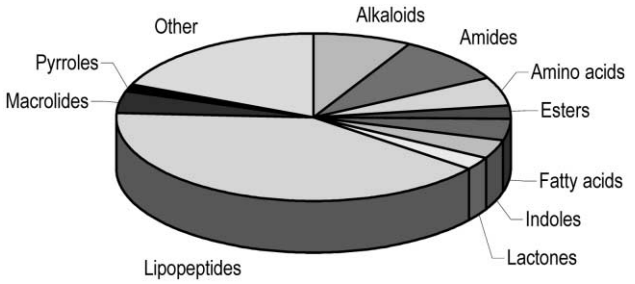


Figure 6. Type of chemical compounds isolated from marine cyanobacteria—2001.[64]

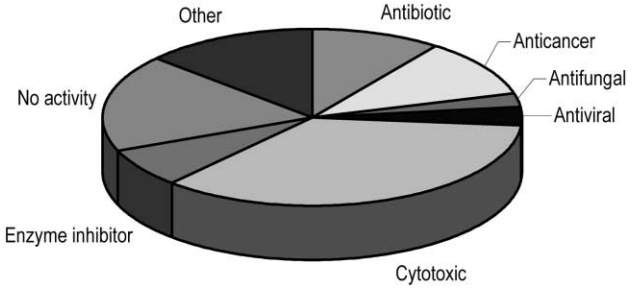


Figure 7. Biochemical activity of lipopeptidic compounds isolated from cyanobacteria—2001.[64]

Lipopeptides are an interesting and extremely biochemically active group of compounds (Fig. 7), with approximately 85% being bioactive. Of which, 41% are cytotoxic being either icthyo-, neuro-, hepato- or endotoxic. The remainder are split between anticancer (antiproliferative) (13%), antibiotic (12%), enzyme inhibitor (8%), antiviral (4%) and antifungal activities (4%). The remaining activity (18%) covers such diverse activities as tumor promoter, herbicide, antimycotic, antimitotic, antimalarial, antimicroalgal, cell-differentiation promoter, cardioactive compounds and sunscreen pigments.

Recently it has been discovered that lipopeptides have an affinity for liposomes and cell membranes, and, due to their low molecular weight, have the ability to pass through the blood-tissue and blood-brain barriers, leading to direct application as drug delivery systems.[228,229] The unique structure of lipopeptides **72**, the presence of an amino acid group and a fatty acid chain, thus being to some extent both hydrophilic and hydrophobic, means that they have the ability to disrupt the function of channel systems found within cell membranes (although, additionally, lipopeptides may be defined as compounds which are *N*-methylated and hence water insoluble, for example didemnin B **26**). Studies have shown that the sequestering of the fatty-acid chain of the lipopeptide within the membrane is an early step of interaction, and might induce the uptake of the lipopeptide into the cell.[230,231] These functions are paramount in controlling intracellular events as diverse as metabolism, contractility, membrane transport, cell division and gene transport. The net result is an increase in phosphorylated proteins and a cascade of subsequent events, a high number of which lead to cytotoxicity (Fig. 7). The authors' have decided to target these secondary metabolites produced by marine cyanobacteria.[227] By studying this group of compounds, the authors thus believe that they are covering a wide variety of biochemical activities, focusing on compounds which can easily be adapted for efficient drug delivery and at the same time streamlining the isolation and screening techniques, thus saving both time and capital.

9. *Lyngbya majuscula* (*Microcoleus lyngbyaceus*)

Members of the Oscillatoriaceae, especially the filamentous cyanobacterium, *L. majuscula* Gomont, known botanically as *M. lyngbyaceus*, have proven to be a rich source of novel and biologically active compounds (Fig. 8). Since 1984, when Carter et al.[232] reported an abundance of unique secondary metabolites from this cyanobacterium, the number of reported novel compounds of cyanobacterial origin continues to increase dramatically. To date,

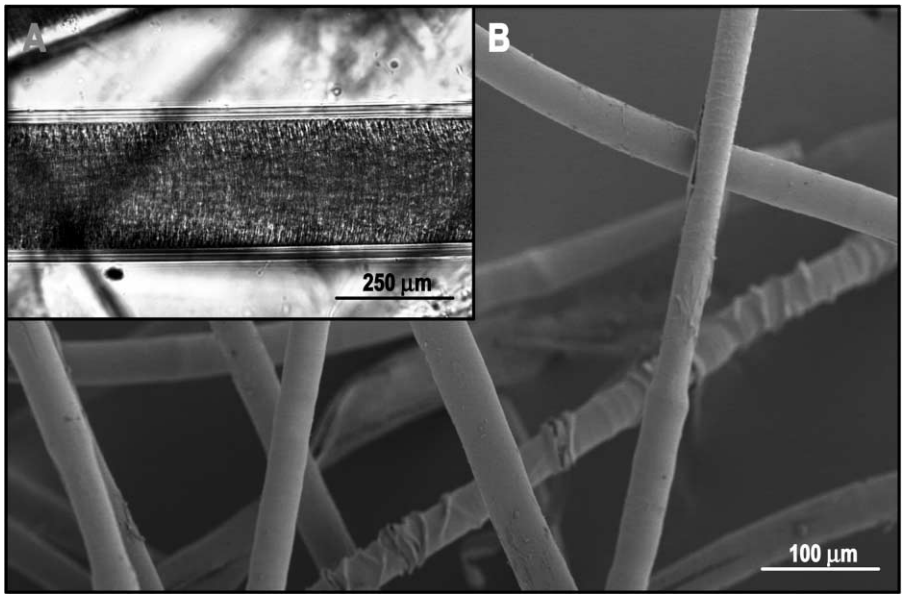
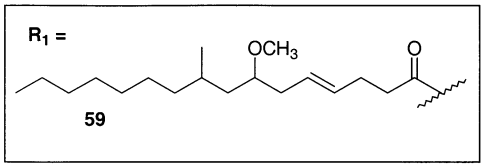
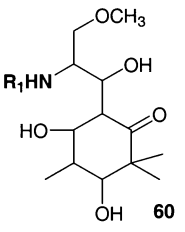
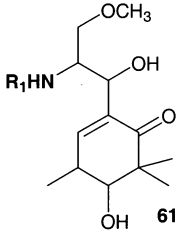
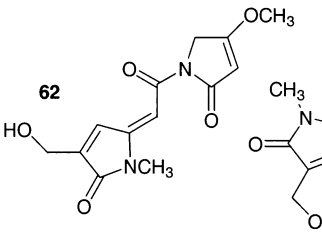


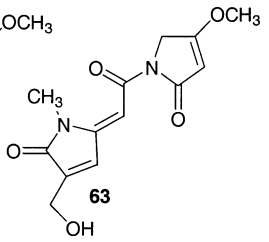
Figure 8. (A) Phase contrast light micrograph (×400) and (B) scanning electron (×10,000) micrograph of a Tahitian strain of *Lyngbya majuscula*.

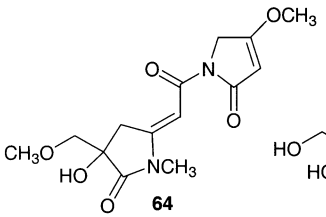


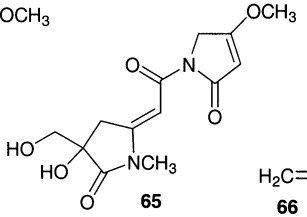


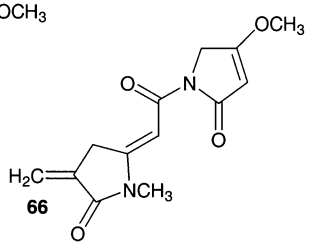


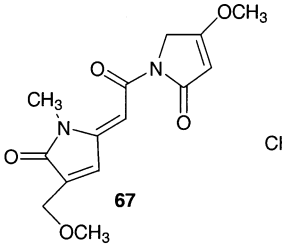


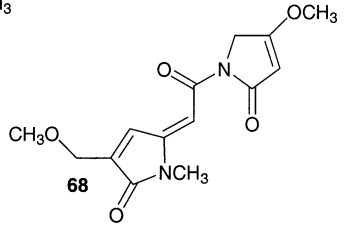


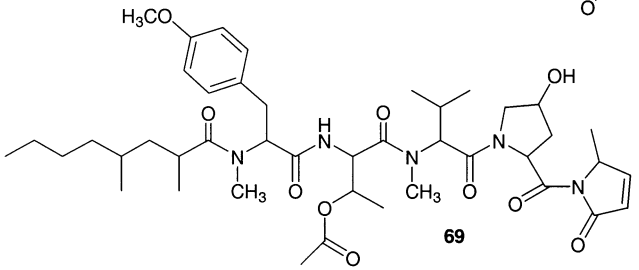


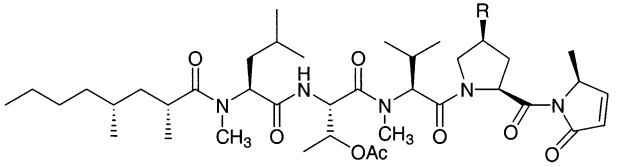


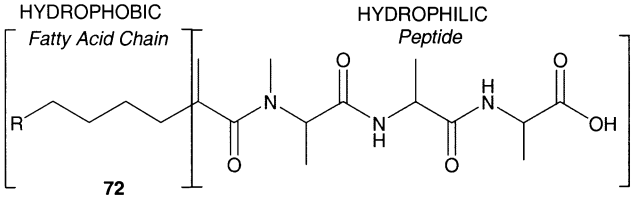


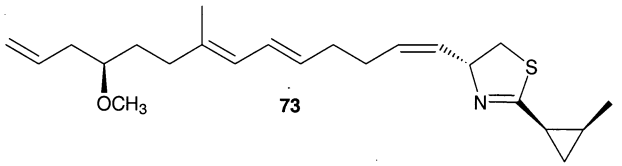












59	7-methoxy-9-methylhexadec-4(*E*)-enoic acid	**66**	Pukeleimide E
60	Malyngamide D	**67**	Pukeleimide F
61	Malyngamide E	**68**	Pukeleimide G
62	Pukeleimide A	**69**	Majusculamide D
63	Pukeleimide B	**70**	Microcolin A
64	Pukeleimide C	**71**	Microcolin B
65	Pukeleimide D	**72**	Stylised lipopeptidic compound
		73	Curacin A

approximately 30% of all natural products isolated from marine cyanobacteria have been isolated from this particular cyanobacterium (Table 1).

L. majuscula is one of the most abundant benthic cyanobacteria in the ocean. This filamentous cyanobacterium has been found throughout the tropical and sub-tropical regions of the world. Novel and biochemically active compounds have been isolated from material collected in Australia,[233] Curaçao,[234] Florida,[23] Grenada,[235] Guam,[64] Hawaii,[9] Indonesia,[236] Madagascar,[69] Marshall Islands,[237] Mozambique,[238] Okinawa,[218] Philippines,[23] Puerto Rico,[239] Tahiti,[227] Venezuela[55] and the Virgin Islands.[59] Interest in this species was first spurred by its ability to induce a dermatitis-like condition in swimmers ('swimmers itch'), which was traced to the potently inflammatory, blister-producing and tumor-promoting compounds aplysiatoxin **10**,[240] debromoaplysiatoxin **11**[45] and lyngbyatoxin A **12**, B and C.[216]

An analysis of the literature for natural products of the marine filamentous cyanobacterium, *L. majuscula*, shows that approximately 75% of the 113 compounds which have been isolated and tested have some sort of biological activity. This includes antibiotic (7%), anticancer (antiproliferative) (14%), antifungal (10%), antiviral (3%), cytotoxic (49%) and several other novel activities, e.g. such as immunosuppressants, tumor promoters, antifeedants and antimitotic agents (17%) (Fig. 9).

It is interesting that such a large number of cytotoxic compounds have been isolated, this perhaps being due to the screening-based isolation techniques that several groups have used in the past. Ecologically, this phenomenon may be explained when it is known that *L. majuscula* has the ability to form toxic blooms. These toxic compounds, together with the antifeedants, tumor promoters and antimitotic agents (Table 2) isolated, would be used to affect the surrounding environment, killing off or discouraging fish and invertebrate grazers and denudifying the local populations of algae, microalgae and cyanobacteria which would compete for the same nutrients. Analysis of the 113 *L. majuscula* compounds isolated to date, shows that 58% are lipopeptides (cyclic or linear) [amino acid-derived compounds], 2% are of pure amino acid composition, 11% are fatty acids, 6% lactones, 5% alkaloids, 6% amides and 12% are pyrroles (Fig. 10). Of these lipopeptidic compounds, 43% are cytotoxic and 22% have no activity, with the remaining 35% spread evenly between antibiotic, anticancer (antiproliferative), antifungal, antiviral and other

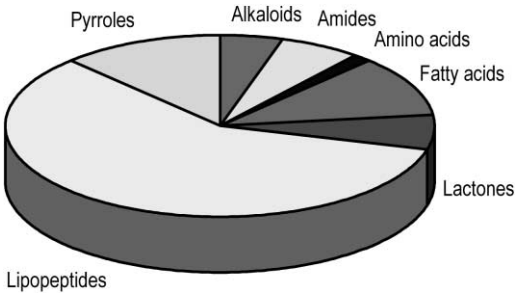


Figure 10. Type of compounds isolated from *Lyngbya majuscula*—2001.[64]

activities (Fig. 11). These figures compare closely with those obtained from analysis of the full complement of marine cyanobacteria-derived compounds (Figs. 6 and 10), although the diversity of the compound types and the various activities is slightly less (Figs. 7 and 11). The authors' believe that the diversity of biological activities found when studying *L. majuscula*, the proven track record of novel compounds discovered and the fact that the collection of this organism is relatively simple and inexpensive, makes this species a perfect choice for the authors' model natural product isolation, cultivation and bioprocess intensification programme.

L. majuscula produces several promising drug candidates which have been studied for the treatment of diseases, such as cancer, acquired immune deficiency syndrome (AIDS), immunosuppression for the prevention of organ rejection, multidrug resistant reversal agents, and antibiotic and antifungal agents (Table 2).[50,61,70,216,219,254] The US NCI has registered two secondary metabolites isolated from the 'algae' *L. majuscula* for further study. The macrolides, debromoaplysiatoxin and anhydro-debromoaplysiatoxin (NCI Nos. 271679 and 694448 (Table 2)), classified as tumor promoters, which target protein kinase C activity, are currently being used to better understand carcinogenesis. Such investigations may facilitate a greater comprehension of the process of human macrophage differentiation. As a result, there are at least 13 associated patents relating to the effect these compounds have on protein kinase modulation (US Patents Nos. 6,043,270, 5,962,498, 5,955,501, 5,936,066, 5,891,906, 5,891,870, 5,886,019, 5,750,568, 5,716,968, 5,643,948, 4,906,451, 4,757,019 and 4,342,751). Several other institutes are also actively studying compounds isolated from *L. majuscula*. Eli Lilly and Company (Indianapolis, IN, USA) together with Richard Moore (University of Hawaii) have been working

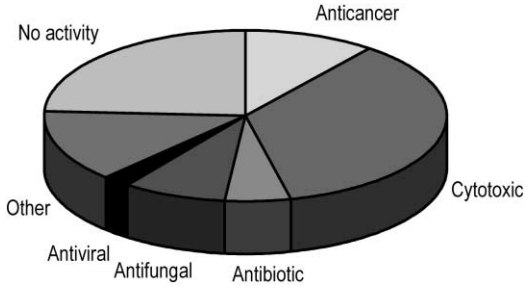


Figure 9. Reported biological activity of *Lyngbya majuscula* compounds—2001 (113 compounds).[64]

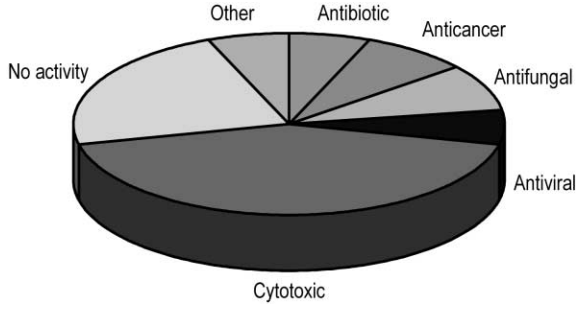


Figure 11. Biochemical activity of lipopeptidic compounds from *Lyngbya majuscula*—2001.[64]

74 Curacin B
75 Curacin C
76 Curacin D
77 Laxaphycin A
78 Laxaphycin B

on the commercialisation of derivatives from majusculamide C (structurally similar to dolastatin 12 **32**), which inhibit plant fungal pathogens, with limited success (US Patent No. 4,342,751).[63] Koehn et al. (Harbour Branch Oceanographic Institute) isolated microcolin A **70**, B **71** and C[63] from *L. majuscula* and, together with several chemosynthetic analogues such as microcolin A_3, have uncovered some startling results. These workers found that, in addition to its immunosuppressive activity,[260] microcolin A **70** and A_3 mediate thymocyte apoptosis (programmed cell death), via a novel mechanism, and cause a significant reduction in mouse T-hydridoma DO11.10 and human G1-101A breast carcinoma cell viability, implying that they may have potential for therapeutic use as antineoplastic agents.[261] Additional studies of these compounds are planned to investigate the molecular mechanism for this action and the relationship between their pro-apototic, immunosuppressive and potential antitumor activities.

The curacin family of compounds (curacin A **73**, B **74**, C **75**, D **76** and several chemosynthetic analogues) represent one of the true success stories of marine cyanobacterial natural product research. In 1994, when curacin A **73** was first isolated from a Curaçao strain of *L. majuscula* and tested for biological activity, it was found to have substantial biological activity against proliferative cells and appeared to have antimitotic activity.[50] Subsequently, curacin B **74**, C **75** and D **76** were isolated from several strains of *L. majuscula*, tested and again showed antiproliferative activity.[74] These promising results lead to curacin A and its analogues being selected for further study by the US NCI (preclinical research) (US Patents Nos. 5,324,739, 60,052,467 and 6,057,348).[262] Through cellular process experiments, it was discovered that this compound caused the accumulation of cells arrested in the G_2/M phase of the cell cycle, and inhibited the binding of radiolabelled colchicine. Despite impressive activity against HeLa and MCF-7 (breast) cancer cells, however, the non-discriminative activity of this compound and its irreversible inhibition caused it to be excluded from further study.[263] Not to be perturbed, Gerwick et al. in a collaborative venture with Molecular Probes Inc (Eugene, OR, USA), released curacin A for use as a molecular probe for investigating the dynamics of microtubule networks (~£200 for 100 μg)[264] during the year 2000.

It is additionally worth considering the 19 different malyngamide-like compounds **44**–**61**, isolated from various strains of *L. majuscula* (Table 2). Although these compounds are generally not bioactive (except malyngamide J **51**—currently selected for in vivo testing due to its unusual profile in the NCI 60-cell line bioassay system),[219,265] the fact that they have been produced by so many different strains from throughout the tropical and subtropical regions of the world,[61,72,217–219,254,265] suggests that they must perform some important function. What that function is, however, remains to be seen. It has also been noted recently that malyngamides Q **54** and R **55**[61] were the first of the derivatives isolated, with altered geometry about C_6 and, together with malyngamide H **34**[66] the first non-chlorine malyngamides, thus suggesting a novel metabolic pathway. Further evidence comes from two intermediates which have been isolated in the synthesis of the malyngamides. Those malyngamides isolated from shallow waters have associated with them the compound, (±)-7-methoxytetradec-4(*E*)-enoic acid **44**,[72,217,257,266] while those isolated from deep sea strains are linked to the compound, 7-methoxy-9-methylhexadec-4(*E*)-enoic acid **59**.[254] The isolation of these intermediates within the malyngamide metabolic pathway may be used in the future as markers to determine what methods this species employs in barotolerance.

10. New avenues of research within this field

Several secondary metabolites, isolated from *L. majuscula* and other cyanobacterial species, possess synergistic activity, i.e. when similar compounds isolated from the same species or strain of cyanobacterium are combined and screened for activity the sum of the activity of the combined extract is greater than that of its individual activities. Laxaphycin A **77** and B **78**, for example, first isolated from the terrestrial cyanobacteria, *Anabaena laxa*,[267,268] and subsequently found to also be present within *L. majuscula*,[249] were noted to exert a synergistic effect, both in their biological activity against *Candida albicans* and in their growth inhibition of lymphoblastic cell lines.[249] This synergistic effect was observable both for drug-sensitive and drug-resistant cell lines. Synergism between two compounds coproduced by the same organism has rarely been described in the literature. The synergistic effect of surfactin on iturin A, two related lipopeptides, isolated from the same strain of *Bacillus subtilis*, was pointed out in 1992.[269,270] The natural occurrence of these lipopeptide associations in the same organism suggests that the complex might be involved in the cell growth regulation of the producer microorganism, or in the cell growth inhibition of competitor microorganisms. It may be speculated here that this phenomenon may function as an antigrazing mechanism, whereby these two compounds, too potent when present singularly within the organism, are stored separately. When this organism is ingested by herbivores, the compounds are mixed, forming a more potent derivative which can then act on the predator. Although few of these synergistic events have been noted to date, the authors' believe that their actual presence in the marine environment has been underestimated. To the best of the authors' knowledge, no natural product screening programme has investigated this phenomenon, and it is, therefore, a useful and potentially novel area of research.

With the difficulty in gaining access to large tracts of biodiversity within natural habitats globally, the potential devastation of marine organisms found to produce natural products of great importance by over harvesting, coupled with the fact that only a small fraction of all microbes present within the marine environment are culturable via conventional methods, means researchers must develop new techniques to produce natural products in unnatural ways.[6,258] Many researchers believe that combinatorial genetic engineering is a prospective remedy to this problem. In addition to offering a secure supply of naturally occurring metabolites, such technologies can be used to produce more diverse chemicals. Although research is relatively new

within this area, with only a few studies published to date, it seems quite likely that it will soon be possible to transfer biosynthetic genes from one organism to another in order to develop more productive organisms, in essence turning a living organism into an intracellular production factory.

Recently, progress has been made in the identification and complete description of the biosynthesis pathway of microcystin production by *M. aeruginosa* PCC 7806. This non-ribosomally-synthesized lipopeptide has an enzymatic organization with a number of unusual structural and enzymatic features and is synthesized via an integrated polyketide-peptide biosynthetic pathway.[271] Tillet et al.[271] were able to identify a cluster of 10 bidirectionally transcribed open reading frames arranged within two operons as being intrinsically involved in microcystin production via gene disruption, involving the use of knock-out mutants and mutant analysis. This research further demonstrates, that metabolites of mixed origin (i.e. from more than one gene) can now be biochemically or genetically characterised. In addition, the same group has undertaken to clone the gene cluster responsible for the biosynthesis of microcystin-LR **7** within *M. aeruginosa* PCC 7806.[271] Following a similar line of research, Gerwick et al. has targeted oxylipins produced by *L. majuscula*, i.e. the curacins **73**–**76**, the oxylipins being derived from polyunsaturated fatty acids through the action of lipoxygenes. Their methodology involved searching public domain genomic databases in order to yield lipoxygenase sequences and they were able to clone just such a gene from *Pseudomonas aeruginosa* PA01.[272] PCR primers were then produced and successfully yielded a region of similarity within *L. majuscula*. Efforts are currently under way to screen a genomic DNA library of *L. majuscula* using this probe.[272]

The study of genomics alone, however, will not provide all the answers. A recent investigation by Anderson and Seilhamer[273] offers evidence that there is not necessarily a tight correlation between the genomic and protein level. The expression of a gene may, therefore, have no definitive relationship to the ultimate expression or abundance of its protein product, emphasising the need to additionally profile protein expression. This process is known as proteomics, and is defined as the entire protein complement expressed by a genome. The aim of proteomics is to profile the proteins from a cellular or tissue source. As with genomics, when this is performed in a comparative manner between secondary metabolite-producing and non-producing strains of cyanobacteria, the hope is that new biosynthetic targets will be identified.[274] A gene to protein to product functionality will hence need to be considered in a quantifiable mechanistic manner to maximize a step change in novel product development.

11. Conclusions

Recent studies have demonstrated that marine cyanobacteria have the ability to produce secondary metabolites unlike those found in any terrestrial species. Cyanobacterial marine natural products chemistry, as a new area of research, is heavily dependent on experimental methods developed for terrestrial microorganisms. Although this approach has provided a good starting point for investigation, the continued application of methods designed for the isolation, culture and chemical investigation of terrestrial species is likely to limit further study to organisms similar to those already isolated. In order to fully exploit the new opportunities available, it will be necessary to develop novel methodologies, such as those mentioned here, that allow the isolation and culture of microorganisms, which produce natural products unique to the marine environment.

Research into marine microbes and cyanobacteria in particular is hampered by the level of knowledge concerning the basic biology and culture techniques for marine-derived organisms, and many more biological and ecological investigations are needed as a prerequisite for the full exploration of the biochemical potential of these organisms. Although current molecular biology techniques allow the study of essentially all members of the marine microbial community, it is not possible for natural products researchers, at present, to generate sufficient biomass to enable successful chemical investigations of all of these organisms. Vast areas of the marine microbial community therefore lie unexplored, at least in the chemical sense and, clearly, the challenge for the future is the development of a better understanding of the conditions needed to isolate and mass cultivate these life forms.

Currently, drug discovery is passing through a phase of reduced interest because of the enormous efforts which are necessary to isolate the active principles and to elucidate their structures. If however, the diversity of chemical structures found in nature with the narrow spectrum of structural variation of even the largest combinatorial library is considered, it can be expected that natural products will become even more important. Marine cyanobacteria have been proven to biosynthesize secondary metabolites of unlimited structural diversity that can further be enlarged by structure modification by applying strategies of combinatorial chemistry. In the future, progress is expected to depend substantially on methods and techniques that accelerate validation of new target genes potentially related to human disorders. Validation can be provided either by functional gene expression in animal models, or by immediate transfer to high-throughput screening in order to get rapid access to a low-molecular mass effector that gives rise to both a drug candidate and target validation.

The authors believe the investigation of the chemical ecology associated with these natural product-producing microbes is essential, and represents the marine biologist's 'why' to go along with the chemist's 'what' and the bioprocess engineer's 'where' and 'how'. The investigation of marine microorganisms as sources of potentially useful natural products has proved to be a promising area of study, which is beginning to produce results. Novel compounds are being discovered and reported daily. Because the marine environment is highly complex, however, a highly interdisciplinary approach is required in order to realize its potential. As one of the two remaining environments (marine and extraterrestrial) which is yet to be fully explored, a greater understanding of this 'inner-space' will

require a close collaboration between marine biologists, microbiologists, geneticists, bioprocess engineers and natural product chemists.

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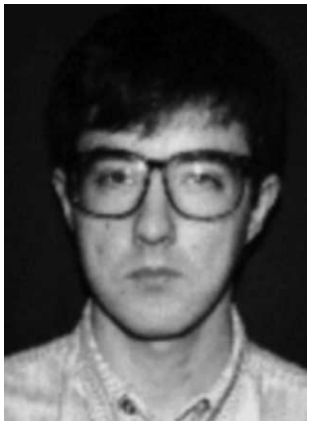
Biographical sketch

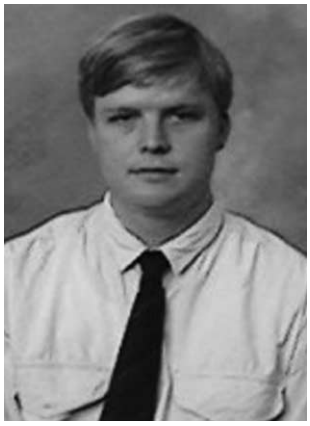
Adam M. Burja received his Bachelor of Science from the University of Melbourne in 1994, completed a post-graduate course in recombinant DNA techniques at the Monash University (1996) and is currently undertaking a Doctor of Philosophy in Biotechnology within the Biochemical Engineering and Environmental Technology Group at Heriot-Watt University. He has several links with industry and academia, was awarded a French Government Scientific Fellowship for 2000 (Australia–France) and is a member of the European Union COST Action for Hydrogen production. His current research interests focus on the effects stress has on marine microorganisms and natural product production, the use of molecular biological techniques within microbial communities for the production of targeted compounds, hydrogen production by microalgae, bioprocess intensification and bioreactor design, marine natural product research and microbial physiology.

Bernard Banaigs completed his Doctor of Philosophy at the University of Montpellier, France in 1983. Upon obtaining his doctorate in Natural Product Chemistry he undertook several post-doctoral positions within internationally renowned institutes, including the Scripps Institution of Oceanography. In 1986 he joined the Centre de Phytopharmacie at the University of Perpignan as a research scientist and is currently chargé de recherché for the Institut National de la Santé et de la recherché médicale. He has strong industrial links with several pharmaceutical companies and is a member of the editorial board of the international journal Marine Biotechnology. He has published extensively and is a regular referee for the journals Botanica Marina and Marine Biotechnology.

Eliane Abou-Mansour is a native of Lebanon where she received her BSc in Chemistry from the Lebanese University at Beirut in 1986. She spent four years teaching high school Chemistry and Physics in Beirut before moving to France, where she obtained her PhD degree in Chemistry of Marine Natural Products in 1994 from the University of Perpignan. She joined the Australian Institute of Marine Science as a Post-doctoral fellow in 1996, working on the chemical properties of natural products from marine microbes. On returning to the University of Perpignan in 1999, she worked on isolation of bioactive compounds from marine cyanobacteria. She joined the Institute of Chemistry at Neuchatel Switzerland in 2000 as a scientific collaborator to work on identifying fungal phycotoxins implicated in trunk and grape vine diseases within the Swiss Role of National Research for Plant Survival and the European Union COST action 835 on plant diseases.



J. Grant Burgess received his Bachelor of Science (Honours) from Queen Elizabeth College, University of London in 1986, and his PhD at Imperial College, London in 1989. Upon completion of his PhD he accepted a post-doctoral research fellowship at the Tokyo University of Agriculture and Technology between 1990–1993 and was appointed Assistant Professor of Marine Microbiology and Biotechnology at the Tokyo University of Agriculture and Technology between 1993–1994. He joined Heriot-Watt University in 1994 as a Lecturer and was subsequently promoted to Reader in Marine Biotechnology in 1999. His main research interests include industrial and medical applications of marine bacteria and fungi.



Phillip C. Wright received his Bachelor of Engineering from the University of New South Wales in 1991, completed a Research Master of Engineering at the University of Wollongong in 1993 and his PhD in 1997 under the direction of Professor Judy Raper. Upon completion of his PhD he took up the post of Lecturer in Chemical Engineering at Heriot-Watt University (1997) and was recently promoted to Reader and is the co-founder of the Biochemical Engineering and Environmental Technologies Group. He has collaborations with industry and academia, is co-chairman of the EPSRC Marine Bioprocess Engineering Network, is a recipient of an EPSRC Advanced Research Fellowship and is a member of the European Union COST Action for Hydrogen production. His current research interests include multi-phase reactor processes, bioprocess intensification, metabolic engineering and protein control and characterization.