Bioactive Marine Natural Products

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Foreword

The chemistry of marine natural products has grown enormously in the last fifty years. On land, communication between insects is largely by pheromones. Because these must be volatile, their chemical structures are often simple and many are easy to synthesize. In contrast, in an aqueous environment communication between living organisms depends on solubility in water. As a consequence, the chemical compounds used in the communication can have complex structures and large molecular weights as long as there is adequate solubility in water.

Since all forms of life are subject to perpetual competition, it is not surprising that the organisms that live in the sea produce an enormous range of biological activity. Besides the compounds that repel predators by their toxicity, there are those which are attractive to make reproduction more probable.

In addition, there is a complex food chain from the simplest organisms to the most complicated. What is edible and what is not is also determined by the secondary metabolites of the life process.

Given all these factors it is not surprising that marine organisms are a wonderful source of biologically active natural products. It has taken half a century for this to be fully appreciated. In this time the means of collection have been developed so that marine diving, at least in shallow coastal waters, is relatively simple. Also, more sensitive biological tests are available and can be carried out on board ship. The result of all this is that there is an avalanche of new and biologically exciting marine natural products. However, there is one negative aspect to this work. It is that the compounds isolated are often available in minute amounts only. Therefore, if the structure is complex, it is an arduous, and often impossible, task to isolate enough of the natural material for clinical trials. This is where synthetic chemistry can come to the help of the clinician. Marine natural products are often wonderful challenges to synthetic chemists.

The present book by Dr. D.S. Bhakuni, a distinguished expert on natural products chemistry, [and Dr. D.S. Rawat] will serve as an excellent introduction to the scientific methods involved in marine natural products chemistry. It includes a description of the compounds and their biosynthesis. Of course, there can be no clinical discovery without prior and extensive biological testing so these

procedures are also described in some detail. But before any clinical tests can be carried out, the compound must be isolated. Even if there is never enough for clinical testing, the isolation and determination of structure must take priority.

All these aspects of marine natural products chemistry are treated with authority in this book. It is certain to become an internationally accepted and widely read volume on an important subject.

D.H.R. Barton (deceased)
College Station
Texas A&M University
TX, USA

Preface

Marine natural products have attracted the attention of biologists and chemists the world over for the last five decades. To date approximately 16,000 marine natural products have been isolated from marine organisms and reported in approximately 6,800 publications. In addition to these publications there are approximately another 9,000 publications which cover syntheses, reviews, biological activity studies, ecological studies etc. on the subject of marine natural products. Several of the compounds isolated from marine source exhibit biological activity. The ocean is considered to be a source of potential drugs.

Marine organisms not only elaborate pharmaceutically useful compounds but also produce toxic substances. One of the most important societal contribution of marine natural products chemists has been the isolation and identification of toxins responsible for seafood poisoning. Outbreaks of seafood poisoning are usually sporadic and unpredictable because toxic fish or shellfish do not produce the toxins themselves, but concentrate them from organisms that they eat. Most marine toxins are produced by microorganisms such as dinoflagellates or marine bacteria and may pass through several levels of the food chain. The identification of marine toxins has been one of the most challenging areas of marine natural products chemistry.

The major occupation of marine natural products chemists for the past two decades has been the search for potential pharmaceuticals. It is difficult to single out a particular bioactive molecule that is destined to find a place in medicine. However, many compounds have shown promise. Marine organisms produce some of the most cytotoxic compounds ever discovered, but the yields of these compounds are invariably so small that natural sources are unlikely to provide enough material for drug development studies.

The art by which marine organisms elaborate bioactive molecules is fascinating. Marine environment provides different biosynthetic conditions to organisms that live in it. Marine organisms generally live in symbiotic association. The pathway of transfer of nutrients between symbiotic partners is of much importance and raises questions about the real origin of metabolites produced by association. A recent trend in marine natural products chemistry is the study of symbiosis. Biosynthesis of bioactive marine natural products provides many challenging problems.

The biological activity of an extract of marine organisms or isolated compounds could be assessed in several ways. Due to limited amount of the material generally available initially and high cost of biological testing, it is impossible in any laboratory to examine all permutation of drug-animal interaction, to unmask the drug potential of a material. Besides, the candidate drug has to pass through rigorous toxicological evaluation and clinical trials before it reaches the clinician's armamentarium. A fair understanding of biological, toxicological and clinical evaluation is essential to those interested in searching potential drugs from marine organisms.

Marine natural products chemistry has passed through several phases of development. The scuba diving made the collection of materials from deep seas easy. Effective methods of isolation provided many potent compounds in pure form. Advancement in instrumentation methods such as nuclear magnetic resonance, mass spectrometric techniques and X-ray diffraction have helped to solve many intricate structural and stereochemical problems. The present text is an effort to fill up the void in bioactive marine natural products. It would be inappropriate to claim that a complete coverage of all bioactive compounds has been made. Attempts have nevertheless been made not to leave out any of the major class of bioactive compounds.

The chemistry and biology of the bioactive metabolites of marine algae, fungi and bacteria and of marine invertebrates; separation and isolation techniques; biological, toxicological and clinical evaluation; bioactivity of marine organisms; biosynthesis of bioactive metabolites of marine organisms; bioactive marine toxins; bioactive marine nucleosides; bioactive marine alkaloids, bioactive marine peptides; and marine prostaglandins are dealt with in separate chapters so that the book may be adopted at any stage by any practicing organic chemist and biologist working in the academic institutions and R&D organizations. Each chapter in the beginning provides highlights of the main points discussed in the text with concluding remarks at the end. References of books, monographs, review articles and original papers are given at the end of each chapter. Considerable progress has been made in the biological evaluation. Thus, marine natural products have drawn organic, medicinal and bioorganic chemists, pharmacologists, biologists and ecologists to work in this area.

The book is dedicated to the late Sir Derek Barton, FRS, Nobel Laureate, Texas, A&M University, USA, who encouraged Dr. Bhakuni to write a book on bioactive marine natural products. The authors are grateful to him for writing the foreword before his sad demise. Thanks are due to the authorities of Central Drug Research Institute, Lucknow, for providing library facilities, and to Dr. S. Varadarajan, FNA, former President, Indian National Science Academy, New Delhi and Prof. John W. Blunt, Department of Chemistry, University of Canterbury, New Zealand for sending interesting information about marine organisms. Thanks are due to Prof. R.S. Verma, Lucknow University, for his valuable suggestions. We thank the publishing staff members of M/s Anamaya Publishers, especially Mr. M.S. Sejwal, who handled the project and offered splendid cooperation.

Finally, one of us (DSB) expresses his sincere thanks to the Council of Scientific and Industrial Research, New Delhi and Indian National Science Academy, New Delhi, for financial support.

D.S. Bhakuni
D.S. Rawat

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Bioactive Metabolites of Marine Algae, Fungi and Bacteria

Abstract

The chapter deals with the bioactive metabolites of marine algae, bacteria and fungi. The chemistry and biological activities of the bioactive brominated compounds, nitrogen heterocyclics, nitrogen-sulphur heterocyclics, sterols, terpenoids and sulfated polysaccharides isolated from marine algae, fungi and bacteria have been reviewed.

1. Introduction

About 30,000 species of algae are found the world over which occur at all places where there is light and moisture and are found in abundance in sea. They supply oxygen to the biosphere, are a source of food for fishes, cattle and man. Algae are also used as medicine and fertilizers. A few algae that excrete toxic substances pollute marine water.

A majority of red algae and almost all the genera of brown algae except *Bodanella, Pleurocladia* and *Heribaudiella* occur in salt water. Many macroscopic green algae like *Codium, Caulerpa, Ulva* and *Enteromorpha* grow in shallow waters. The species of some genera, for example *Prasiola, Enteromorpha* and *Cladophora* grow both in fresh water and sea water. In sea water, many algae grow as phytoplankton (especially the dinoflagellates and certain blue-green algae). Other marine algae grow as benthos, epiphyte on other algae, parts of higher plants, rocks, stones, gravels, sand and mud. A small group of algae occurs in brackish water.

2. Secondary Metabolites of Marine Algae

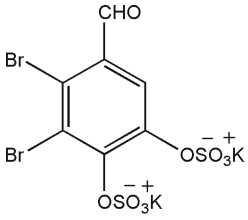
Extensive work has been done on the secondary metabolites of marine algae.[1] The work carried out on *Laurencia* species,[2] blue-green algae[3] and dinoflagellates[4] have been reviewed. Reports are available dealing with amino acids from marine algae,[5] guanidine derivatives,[6] phenolic substances,[7] bioluminescence,[8] carotenoids,[9] diterpenoids,[10] biosynthesis of metabolites,[11] indoles,[12] bioactive polymers[13] and halogenated compounds.[14,15]

3. Bioactive Metabolites

Chemically the bioactive metabolites of marine flora include brominated phenols, oxygen heterocyclics, nitrogen heterocyclics, sulphur nitrogen heterocyclics, sterols, terpenoids, polysaccharides, peptides and proteins. The chemistry and biological activities of the compounds isolated have been reviewed.[16]

3.1 Brominated Phenols

The green, brown and red algae had been extensively analyzed for antibacterial and antifungal activities. The active principles isolated from *Symphyocladia gracilis, Rhodomela larix* and *Polysiphonia lanosa* were: 2,3-dibromobenzyl alcohol, 4,5-disulphate dipotassium salt (**1**), 2,3-dibromo-4,5-dihydroxybenzaldehyde (**2**), 2,3-dibromo-4,5-dihydroxybenzyl alcohol (**3**), 3,5-dibromo-p-hydroxybenzyl alcohol (**4**) and the 5-bromo-3,4-dihydroxybenzaldehyde (**5**). Virtually nothing is known about the physiological importance and the mechanism of biosynthesis of the bromo phenols. Their antialgal activity suggests that they may play a role in the regulation of epiphytes and endophytes. The bromo phenols may be biosynthesised through the shikimate pathway, and bromination may occur in the presence of suitable peroxide.[17]



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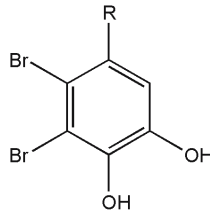
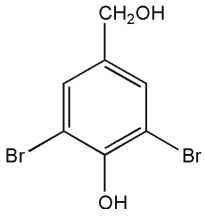


2, R = CHO
3, R = CH_2OH



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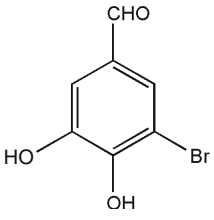

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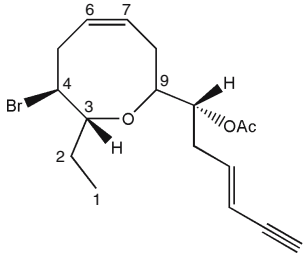
3.2 Brominated Oxygen Heterocyclics

The red algae *Laurencia* sp. have produced the diverse class of natural products.[18–22] *L. glandulifera*[19] and *L. nipponica*[23] had furnished two brominated oxygen heterocyclic compounds, laurencin (**6**)[22] and laureatin (**7**)[23], respectively. Laurencin ($C_{17}H_{23}BrO_3$), m.p. 73–74°C; $[\alpha]_D$ + 70.2° ($CHCl_3$) was isolated from the neutral fraction from methanol extract of the algae. The IR of the purified compound suggested the presence of a terminal methine (ν_{max} 3285 and 2180 cm^{-1}), an acetoxyl (1735 and 1235 cm^{-1}) and an ether (1168 and 1080 cm^{-1}) functions and *trans* and *cis* double bonds (3040, 950 and 750 cm^{-1}). The UV (in EtOH), λ_{max} 224 nm (ε 16,400) and 232 nm (ε 11,000) showed the presence of a conjugated diene or enyne. The NMR spectrum of the compound indicated the presence of four olefinic protons and an acetoxyl and ethyl groups. The presence of ethyl group was confirmed by isolation of CH_3—CH_2—CHO on ozonization of laurencin.



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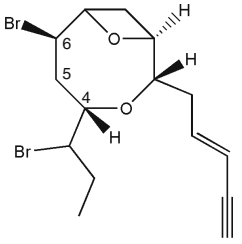

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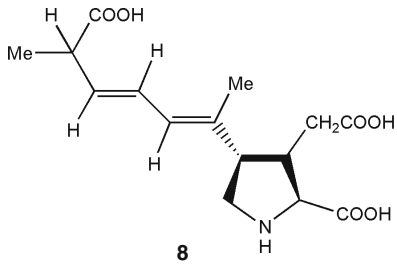
Laurencin consumed four moles of hydrogen over platinum in ethyl acetate to yield octahydrolaurencin ($C_{17}H_{31}BrO_3$). On mild hydrolysis with KOH laurencin gave deacetyl laurencin ($C_{15}H_{21}BrO_2$) which was reconverted into original ester in good yield by treatment with acetic anhydride/pyridine. Reduction of octahydrolaurencin with $LiAlH_4$ afforded a debromoalcohol ($C_{15}H_{30}O_2$). Extensive NMR studies and spin decoupling experiments of the parent compound and the degradation products established structure **(6)** for laurencin.

Laureatin ($C_{15}H_{20}Br_2O_2$) m.p. 82-83°C; $[\alpha]_D$+ 96° (CCl_4) has been isolated from the Japanese seaweed.[18] UV absorption λ_{max} 223 nm (ε12,800), 229 nm (ε10,400) and IR peaks at ν_{max} 3300, 2100, 1140, 1045, 975 and 965 cm^{-1} indicated that laureatin is an ether having a conjugated enyne group and contains neither hydroxyl nor carbonyl functions. NMR and spin decoupling experiments confirmed the presence of —CH_2—CH=CH—C≡CH and —$\underset{|}{CH}$—CH_2—CH_3 groups. NMR spectrum of the compound also contained peaks for 6 protons at τ 5.0, 6.5; three one-proton septets at τ 5.12 and 5.87, a broad quartet at 5.62 and two multiplets centered at 6.2 and 6.35. These absorptions were ascribed to protons on carbons bearing an ether oxygen or a bromine atom. Laureatin consumed three moles of hydrogen over platinum catalyst in ethanol to yield hexahydrolaureatin. On treatment with zinc in refluxing acetic acid and then with dilute alkali hexahydrolaureatin

gave an unsaturated glycol. Laureatin was finally assigned structure (**7**) on the basis of chemical degradation studies and NMR spectroscopic data. Other brominated metabolites which have been isolated from *Laurencia nipponica*, are prelaureatin, laurallene, isolaurallene, bromofucin, and chlorofucin. The total syntheses of (+)-prelaureatin and (+)-laurallene have been achieved recently.[24] Laureatin (**7**) and isolaureatin exhibit significant larvicidal activity (IC_{50}) 0.06 and 0.50 ppm, respectively, in mosquitos. Brominated compounds isolated from marine algae, particularly bromophenols, are toxic and due to this they are not of clinical value.

3.3 Nitrogen Heterocyclics

Marine algae had yielded nitrogen containing heterocyclic compounds. Of these the most interesting compounds are domoic acid (**8**) and the kainic acid.



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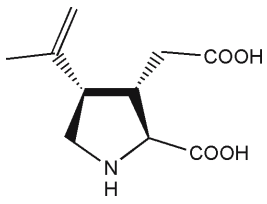
Domoic acid (**8**) ($C_{15}H_{21}NO_6$), m.p. 217°C (dec.): $[\alpha]_D$ – 109.6° [H_2O] an anthelmintic agent was first isolated from the alga *Chondria armata*.[25-29] The acid had UV λ_{max} 242 nm (log ε 4.42). Catalytic reduction of the compound with Pt-O_2 gave tetrahydrodomoic acid. Acetylation of the compound gave N-acetyl derivative, m.p. 121°C; $[\alpha]_D$–56° [H_2O]; λ_{max} 242 nm (log ε 4.48). Domoic acid showed marked anthelmintic activity. It was found to be very effective in expelling ascaris and pinworms without any observable side effects.

3.4 Kainic Acids

In Asia, the dried red alga *Digenea simplex* is widely used as an anthelmintic. It is found very effective in the treatment of ascariasis.[30] In the Mediterranean, extract of the alga *Corallina officinalis* is also used for the same purpose. Kainic acids as the active principles had been isolated from these algae. Of the kainic acids, α-kainic acid was the most active constituent. The structure (**9**) for α-kainic acid had been assigned by degradation studies[31] and confirmed by its synthesis.[32] The stereochemistry of α-kainic acid is shown in (**9**).[33]



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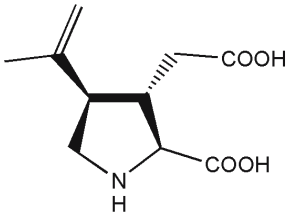
Isomers of α-kainic acid had been isolated from alga *Digenea.* The isomers isolated are γ-allo-kainic acid (**10**)[34] and γ-kainic acid lactone (**11**).[35] L-α-kainic acid and L-α-allo-α-kainic acid are configurational isomers. In α-kainic acid the substituents at C-2 and C-3 and at C-3 and C-4 are *trans* and *cis*, respectively. In α-allokainic acid configurations at both the centres are *trans*. α-Kainic acid lactone was considered to be an artifact.[36] α-Kainic acid had been found effective in the treatment of ascariasis, with a single dose of 5 to 10 mg per adult resulting in a 40 to 70% reduction in the population of instestinal parasitic worms. α-Allokainic acid was found to have far less anthelmintic activity. Several preparations of kainic acids are available in the market, including 'Digenin' and 'Helminal' (The Merck Index, 1968). This represents one of the few instances in which clinically useful pharmaceutical product has been isolated from marine source.



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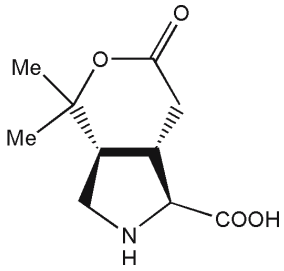

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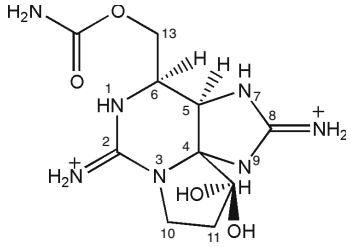
3.5 Guanidine Derivatives

Certain shellfish periodically become poisonous to humans. It is now well established that the substance responsible is produced by a marine plankton, *Gonyaulax catenella*. At certain unpredictable time the red plankton multiply and cause "red tide". Although many fishes are killed by this "red tide", mussels and clams survive and concentrate the toxic principles, thus becoming poisonous to humans. The toxin isolated from the Alaskan butter clam, *Californian mussel*[37] and the alga *Gonyaulax catenella*[38-40] is called saxitoxin (**12**).



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Saxitoxin ($C_{10}H_{19}N_7O_4$) when heated with P/HI in acetic acid, gave weakly basic compound I, $C_8H_{10}N_2O$ (m.p. 100-102°C), NMR analysis of I indicates

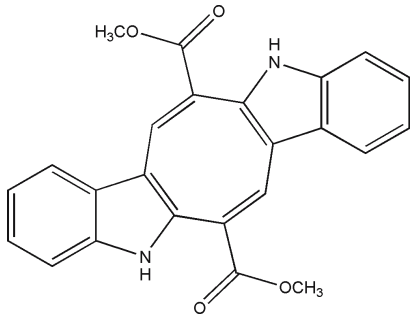
the presence of one C—CH_3 group.[41,42] On oxidation with potassium permanganate, urea and guanidinoacetic acid were obtained. Hydrogenation of I in the presence of platinum oxide (200 mole % hydrogen absorption) gave II, $C_8H_{14}N_2O$ (m.p. 129-131°C) which also contained one C—CH_3 group. Strong acid hydrolysis of II led to the strongly basic, and highly hygroscopic oily diamine III, $C_7H_{16}N_2$ and on heating with Pd-C, III resulted in the formation of a substance which readily gave a positive Ehrlich test for pyrroles. On the basis of these data, it was concluded that III was a pyrrolidine and II was a saturated cyclic urea. This conclusion was fully supported with its ultraviolet absorption and its strong infrared absorption at 3410 and 1635 cm^{-1} in chloroform. The structure (**12**) to saxitoxin was assigned on the basis of degradation studies and spectroscopic analysis. Saxitoxin blocks nerve conduction by specifically interfering with the intital increase in sodium permeability of the membrane. The symptoms caused by the toxin include peripheral paralysis. In extreme cases, complete loss of strength in the muscles and finally death occurred which is caused due to respiratory failure.[43] Saxitoxin is absorbed from the gastro-intestinal tract. It produced no major vascular action. The oral LD_{50} for toxin in various species of animals is reported. In man death had occurred following ingestion of as little as 1 mg of toxin.[44] The toxic compounds from marine algae appear to have biomedical potential. The compounds with neurotropic effects may yield important drugs.

3.6 Phenazine Derivatives

The marine alga *Caulerpa lamourouxii* is widely distributed in the Phillippines. The upper branches are eaten as a 'salad', despite their peppery and astringent taste. However, the alga is found toxic to some individuals. Chemical investigation of the alga had furnished caulerpicine, caulerpin, cholesterol, taraxerol, β-sitosterol and palmitic acid.[45] Caulerpin had also been isolated from *Caulerpa sertularioides, C. racemosa* var. *clavifera*[46] and caulerpicin from *C. racemosa*.[47]

Caulerpin (**13**) ($C_{24}H_{18}N_2O_4$) (M^+ 398) red prisms m.p. 317°C had λ_{max} 222, 270, 292, 317 nm (ε 50,000, 27,000, 29,000 and 35,000); IR bands at 1684, 1631 and 1613 cm^{-1} suggesting the presence of carbonyl functions in conjugation with aromatic system. The NMR spectrum of the compound indicated the presence of 18 protons τ 6.17 (6H, 2 OMe), 2.4-3.0 (8H, m), 1.76 (2H, s) and 1.36 (2H, s). Aromatic protons signal at τ 2.4-3.0 and 1.79 and the IR bands at 730 and 920 cm^{-1} suggested the presence of two identically substituted aromatic ring systems. This was substantiated by elimination of 26 mass units (CH=CH) in the mass spectrum of caulerpin, caulerpinic acid and decarboxy caulerpin acid. Caulerpin contained two methoxy groups in the form of α,β-unsaturated methyl ether group [ν_{max} 1685 cm^{-1}; NMR τ 6.17 (6H)]. Its mass spectrum supported the assignment m/z 398 (M^+), 366 (M^+–MeOH), 338 (366–CO), 306 (338–MeOH), 339 (M^+–CO_2Me) and 280 (M^+–$2CO_2Me$). The M^+ peak in the mass spectrum was the base peak.

Saponification of caulerpin with alcoholic KOH yielded caulerpinic acid ($C_{22}H_{14}N_2O_4$) (M^+ 370). The two exchangeable protons at τ 1.36 were due to secondary amino groups. The functions were conjugated with the two methoxy carbonyl groups as indicated by the low frequency carbonyl absorption (1685 cm^{-1}). The methoxy carbonyl groups were placed at the two α-positions of the two naphthalene rings conjugated with the NH groups at the β-positions. This arrangement accounted for the strong hydrogen bonding of the –NH protons. Caulerpinic acid when heated with copper bronze in quinoline at 200-210°C yielded a decarboxylated compound m.p. >300°C, (M^+ 282). On the basis of these studies caulerpin was assigned the structure α,β-dihydrodibeno[b,i]phenazine-5,12-dicarboxylate (**13**).[48] The stability of the compound was stated to favour the linear structure rather than the geometrical isomer. Caulerpin caused a mild anesthetic action when placed in the mouth, which resulted in numbness of the lips and tongue. In some people it produced toxic effects. The toxic syndrome had been reported to be some what similar to that produced by ciguatera fish poisoning.



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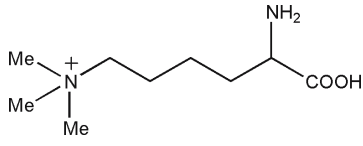
3.7 Amino Acids and Amines

Extracts of the marine algae *Laminaria angustata* and *Chondria amata* are reported to contain agents with hypotensive and other pharmacological properties. Laminine (**14**), a choline like basic amino acid had been isolated from a number of marine algae.[49,50] The compound had been characterised as trimethyl(5-amino-5-carboxypentyl)ammonium oxalate (**14**). Several syntheses of laminine are reported.[51]



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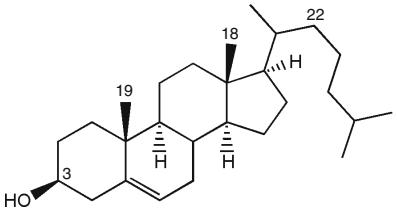
Laminine was isolated from water extracts of *Laminaria angustata* by amberlite ion exchange resin, IR-120 in acidic form and subsequent formation of reineckate and oxalate salts. The other amino acids isolated from this

source were: L-lysine, L-arginine, ethanolamine and choline. Laminine monocitrate was found to have a transitory hypotensive effect. Laminine, in general, depressed the contraction of excited smooth muscles. Laminine monocitrate had an LD_{50} in mouse, 394 mg/kg. It is considered to be a potentially useful pharmacological agent. Steiner and Hartmann[52] had reported the widespread occurrence of volatile amines, such as methylamine, dimethylamine, trimethylamine, ethylamine, propylamine, isobutylamine, isoamylamine, 2-phenylethylamine and 2-methylmercapto propylamine in red, green and brown algae. It is mentioned that biological activities of some of the extracts of the marine algae may be due to the presence of these amines.

3.8 Sterols

The presence of sterols in algae was first established by Heilbron et al[53] and later by Tsuda et al.[54] Gibbons et al[55] established the presence of 22-dehydrocholesterol and demosterol in red algae. However, later investigations showed that the sterol content of red algae were more varied than had been believed.[56] Idler et al[57] examined some species of red algae and found that the three species contained C_{27}, C_{28} and C_{29} sterols. An interesting feature of their result was the considerable variation in sterols content of four different samples of the alga *Rhodymenia palmata*. The percentage of demosterol, for example, varied from 97.2 to 7.7% in the mixture of sterols. Similary, cholesterol was detected in the concentration as high as 97.3% and as low as 2.1%. Cholesterol was again found the major sterol of Rhodophyta. Four species of algae, *Rhodymenia palmata, Porphyra purpurea, P. umbilicalis* and *Halosaccion ramentaceum* were found to contain desmosterol as the main sterol. However, *Hypnea japonica* was the only alga having 22-dehydrocholesterol as the major sterol. Of the 34 algae investigated by the Japanese and British investigators, only one sterol was detected in 25 species, while nine were found containing two sterols. Meunier et al[58] had given a comparative data of 14 species of Rhodophyta. All the species examined were found to contain cholesterol (**15**) as the major sterol except *Hypnea musciformis* in which 22-dehydrocholesterol (**16**) was detected in the highest concentration. *Hypnea japonica* was another example in which 22-dehydrocholesterol was present as the major sterol.



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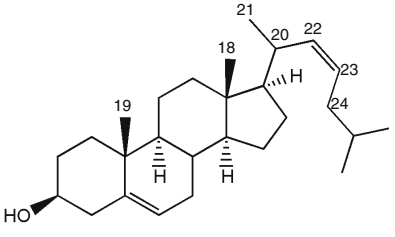

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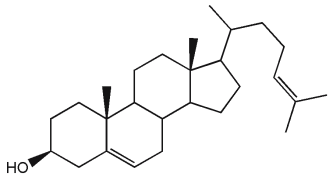
The distribution of sterols in algae had been reviewed.[58,59] Red algae contained primarily cholesterol (**15**). Several species contained large amount of demosterol (**17**), and one species contained primarily 22-dehydrocholesterol. Only a few rhodophyta contained traces of C_{28} and C_{29} sterols. Fucosterol (**18**) was the dominant sterol of brown algae. Most phaeophyta also contained traces of cholesterol and biosynthetic precursors of fucosterol.



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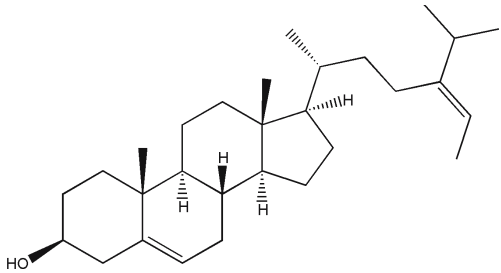

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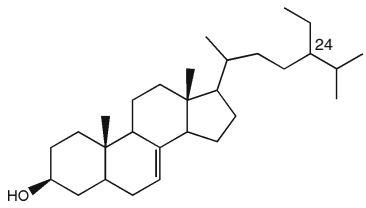
The sterols of green algae were much more varied. The green algae contained chondrillasterol (**19**), poriferasterol (**20**), 28-isofucosterol, ergosterol and cholesterol.



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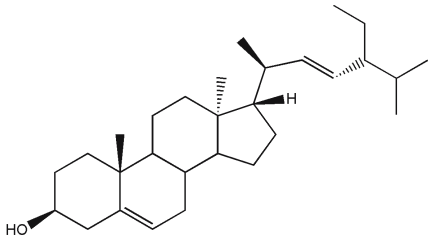

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24-Methyl cholesterol and sargasterol differ from fucosterol (**18**) in that the double bond is shifted to the C-28 position and is saturated at position 24. Sargasterol and fucosterol are isomers. The methyl group at position 20 in the former is α-oriented, whereas it is β-oriented in the latter. The sterols from marine algae are reported to be non-toxic and have the ability to reduce blood cholesterol level. They are also reported to reduce the tendency to form a fatty liver and excessive fat deposition in the heart.[60]

3.9 Sulfated Polysaccharides

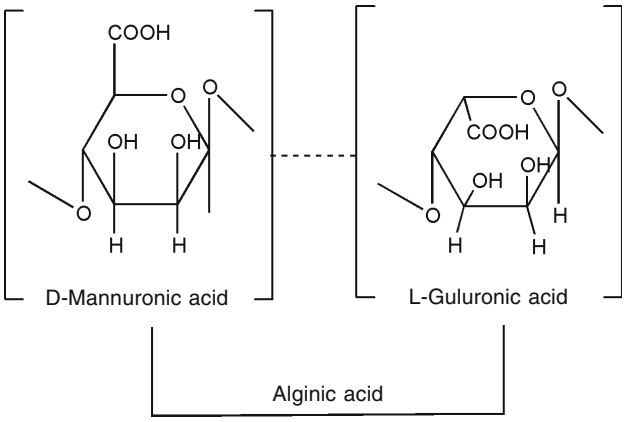
The sulfated polysaccharides obtained from seaweeds are economically most important products due to their extensive use in food and medicine. Of the four major seaweed classes, the rhodophyceae (red algae), the phaeophyceae (brown algae), the cyanophyceae (blue-green algae) and the chlorophyceae (green algae), the first two classes produce polysaccharides of main interest. The red algae produce carrageenan, agar, agarose, furcellaran or Danish

agar. Alginic acid is obtained from brown algae. The use of seaweed extracts in food and medicine is reviewed.[61] Carrageenan are produced by species of *Chondrus, Eucheuma, Gigartina* and *Iridea.* There are different views on the structure of red seaweed polysaccharides.[62] It is generally suggested that carrageenans be defined as a polysaccharide comprising D-galactose units and derivatives linked alternatively α (1→ 3) and β (1→ 4). The ι, κ, λ and μ and other carrageenans represent variations of this primary and general form in which the galactose units are sulfated in a definite pattern and/or are present in the 3,6-anhydro form expressed generally as an A-B-A polymer. Pernas et al[63] however, do not agree on the validity of the above simplified structural approach to carrageenan. These workers believe that carrageenan is a continum of potassium precipitable material of continuously variable structural form. The ester sulphate groups are distributed randomly on all available hydroxyl groups in κ, in support of this hypothesis. The chemical structure of κ and λ carrageenans are still a matter of discussion. κ Carrageenan is precipitated from dilute solution with potassium ions, and is believed to consist primarily of alternating anhydrogalactose and sulphated galactose units linked α 1,3 and β 1,4. λ Carrageenan contains little anhydrogalactose. It consists chiefly of mono- and disulphate galactose units with, perhaps, the same alternating 1,3 and 1,4-linkages. Both κ and λ carrageenans are reported to be strong antigens.[64] The latter is more potent than the former. In general, they behave as typical carbohydrate antigens. λ Carrageenan is also reported to stimulate the growth of connective tissues.[64]

Chondrus crispus and *Gelidium cartilagineum*, the well-known sources of carrageenan and agar, respectively, had been found to possess antiviral properties attributed to the galactan units in the polysaccharides of both. The specific antiviral activity had been shown against influenza B and mumps virus in embryonated eggs even after 24 h inocubation. Carrageenan was also found as anticoagulant and antithrombic agent. The use of carrageenan in ulcer therapy had been studied intensively. It was thought at first that the polysaccharide inhibits the activity of pepsin and that its action in preventing ulcers was due to this property.[65] However, subsequent studies revealed that the polysaccharide plays a much more active role than enzyme inhibition. In fact, it was found that *in vivo*, pepsin was not inhibited by carrageenan. The polysaccharide reacts with the mucoid lining of the stomach and gives a protective layer through which pepsin and acid have difficulty in passing. The treatment of gastric and duodenal ulcers by carrageenan was enjoying considerable popularity in France and Great Britain. In many cases of ulcer carrageenan proved an effective cure.[66]

Alginic Acid

This polysaccharide is obtained from the brown seaweeds, especially from species of *Fucus* and *Macrocystis*. Chemically alginic acid (**21**) is made up of two monomers, the D-mannuronic acid and L-guluronic acid. Both these



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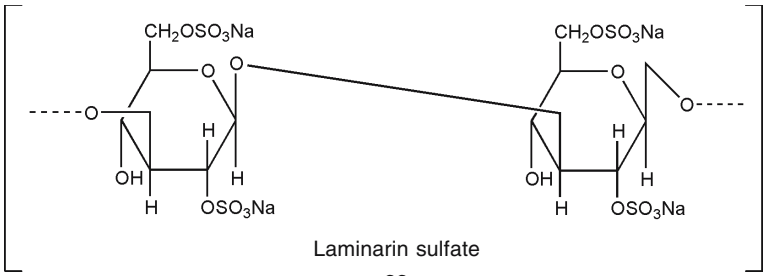
sugar acids are stereoisomers and differ only in the configuration of the carboxyl group. The two uronic acid moieties in alginic acid are linked though β-1,4-glycosidic linkage in such a way that the carboxyl group of each unit is free, while the aldehydic group is sheilded by the glycosidic linkage. Biosynthesis of alginic acid is not yet known. An attractive hypothesis of its formation from guanosine diphosphate mannose has been proposed.[67]

Commercially, sodium alginate is extracted from giant brown seaweed (*Macrocystis pyrifera*), horsetail kelp (*Laminaria digitata*) and sugar kelp (*Laminaria saccharina*). Sodium alginate has been used mainly in the manufacture of ice cream where it serves as a stabilising colloid. It is also used in cosmetics and pharmaceuticals.[68] Calcium alginate is reputed to be a hemostatic agent which stimulates the clotting of blood *in situ* which is subsequently absorbed in the tissue.[69] Sodium alginate is reported to be a useful adjuvent in immunisation against two strains of influenza virus. Sodium alginate is also found effective in diminishing hyper calciuria in urolithiasis, and found useful in the treatment of esophagitis.

The most significant property of sodium alginate is the ability to remove strontium 85 and strontium 87 from the body without seriously affecting the availability of Ca, Na or K in the body.[70] This selective action of sodium alginate is of great potential to remove Sr-90 contamination due to fall out from atomic explosions.

Laminarin

It is essentially a linear polymer of β-1,3-glucan, with occasional branching points at C-6 and with a variable proportion of the glucose chains terminated at the potential reducing end with a molecule of mannitol, which can be sulphated to produce a compound with anticoagulant properties. Laminarin (**22**) occurs at certain times of the year to the extent of 35% of the dry weight of *Laminaria cloustoni*. It has been found that laminarin sulphate formed with two sulphate groups by glucose unit gives maximum stability and



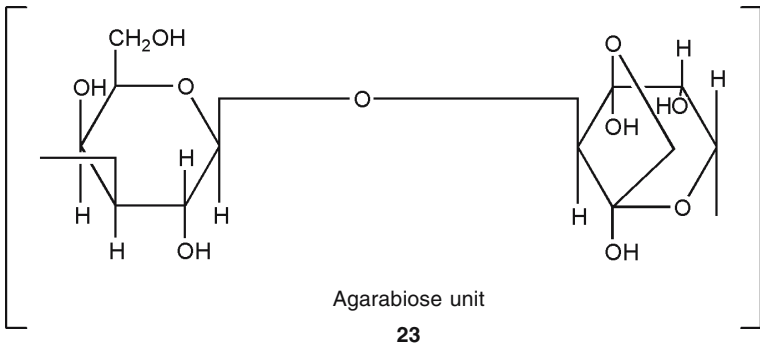
Laminarin sulfate

22

anticoagulant activity. Two lower sulphated laminarins are also reported to have antilipidemic activity like that of heparin.[71,72]

Agar and Agarose

The red algae are the source of agar and agarose. Although these polysaccharides have no direct medicinal use, their use in biomedical research is well known. The genera *Gelidium, Gracilaria, Acanthopeltis* and *Pterocladia* of the Rhodophyceae are the main producers of these materials. Commercial agar generally contains 50-90% recoverable agarose. The structure of agarose was determined by Araki[73] and substantiated by others. Chemically, agarose is a linear polymer made up of repeating units of agarobiose (**23**) which, in turn, consists of a molecule of β-D-galactopyranose attached $1 \rightarrow 4$ to a molecule of 3,6-anhydro-L-galactose. These repeating units are linked $1 \rightarrow 3$ to form the agarose polymer. The presence of traces of sulphate and uronic acid residues have, thus far, been attributed to contamination by agaropectin. Many uses of agarose are described.[74] However, its use in immunology is most interesting.



Agarabiose unit

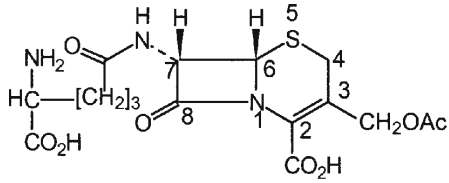
23

The interest to investigate the role of the polysaccharides in the body is growing. The sulphated seaweed polysaccharides are, in some ways, very much like the sulphated mucopolysaccharides of the body and yet, in some ways, are quite different. It is believed that, in some cases, the body may not distinguish the seaweed polysaccharides from those natural to it. In some other cases, they may be so much alike that reactions are started with them, but not finished in the normal manner, which may allow their use as inhibitors.

4. Marine Bacteria and Fungi

Bacteria and fungi are prime producers of the antagonistic substances in terrestrial environment. A similar role in the oceans from these organisms is expected. Indeed, this had been found true. Antibiotic, antiviral, antifungal, and antiyeast activities of these organisms had been reported.[75] Besides, a few growth stimulant properties which may be useful in studies on wound healing, carcinogenic properties, and in the study of cancers are reported.

Among the many bacteria showing antimicrobial activity, a variant of the ichthyotoxic *Pseudomonas piscicida*[75] exhibited marked antagonism to various micro-organisms. A red coloured bacterium obtained from Puerto Rico was found to excrete vitamin B and antibacterial substances into the sea water.[76] The bacteria and fungi from sea are also reported to produce substances which affect central nervous system (CNS), respiratory system (RS), neuromuscular system (NMS), autonomic nervous system (ANS), cardiovascular system (CVS) and gastrointestinal system (GI).[77] Some of the substances are known to produce local effects such as pain, necrosis, edema, parasthesias, pruritis etc. A marine isolate of the fungus *Cephalosporium acremonium* obtained from the sea near a sewage outfall of the coast of Sardina had been reported to produce a number of antibiotic substances.[78] A penicillinase sensitive antibiotic substance named antibiotic N active against Gram-negative bacteria, had been isolated from this source. This material was reported to be cephalosporin C (**24**)[79-81] which was different from cephalosporin N.



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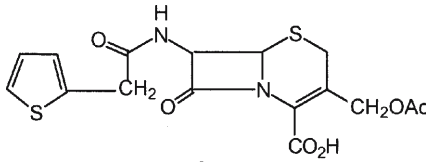
Other antibiotic substances isolated from *C. acremonium* were found to be penicillinase resistant and active against Gram-positive bacteria.[78] These substances were named cephalosporin P. This organism was also found to be a source of cephalothin (**25**), a semisynthetic derivative of cephalosporin C, having antibiotic action similar to that of benzylpenicillin but insensitive to penicillinase. It was active against a number of penicillin resistant *Staphylococcus* and some Gram-negative species of bacteria. A number of chemically-related antibiotic substances named cephalosporins P_1, P_2, P_3, P_4 and P_5 had been isolated from the marine species of fungus *Cephalosporium acremonium*.[82]

Cephalosporin P_1 ($C_{33}H_{52}O_8$) m.p. 147°C; $[\alpha]_D$ + 28° [$CHCl_3$] was a mono-basic triterpenic carboxylic acid. Methylation of the acid with CH_2N_2 at 0°C gave a monomethyl ester m.p. 196°C, while methylation at room temperature with CH_2N_2 produced a product containing nitrogen. The acid



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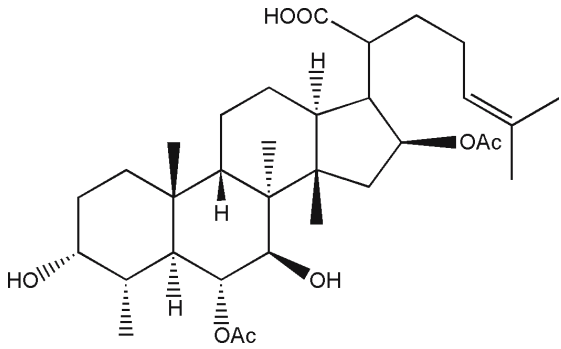
and its methyl ester rapidly absorbed one mole of hydrogen in the presence of PtO_2 to give dihydrocephalosporin P and dihydrocephalosporin methyl ester, respectively. On standing in 1N NaOH at 37°C cephalosporin P lost an acetyl function to give a hydroxy acid m.p. 220°C. Besides, hydrolysis with alkali yielded a product which rapidly lactonised on acidification to give a neutral compound, m.p. 186°C. The chemical studies when combined with NMR and mass spectral data, structure (**26**) was assigned to cephalosporin P_1.[83] It exhibited good activity against *Bacillus mesentericus, Mycobacterium phlei* and *S. aureus in vitro*.[83]



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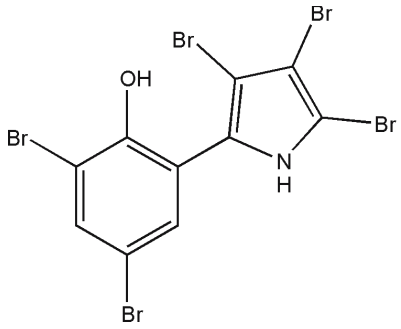
Several bacteria, which produce antibiotic substances, had been isolated from the shallow water near Puerto Rico. A bromo pyrrole antibiotic has been isolated from *Pseudomonas bromoutilis*,[84] which showed activity against many Gram-positive bacteria (at levels of 0.06 mg/mL in broth assay test), but was not active by the subcutaneous route in mouse protection tests. The bromo compound (**27**) ($C_{10}H_4Br_5NO$) was unique in that over 70% of its weight consisted covalently bonded bromine. The mass spectrum of the compound suggested a molecular weight 553.5 and the presence of five bromine atoms from the clasture of isotope peaks. A preferential and sequential loss of one, two and three bromine atoms from the molecular ion together with loss of HCN was observed. Metastable ion peaks corresponding to a simple cleavage of the phenol and pyrrole portions were also discernible. The structure (**27**) for the antibiotic was finally established by X-ray crystallographic analysis[84] and confirmed by its synthesis.[85] Pyrrolnitrin (**28**), a chloropyrrole had been isolated from *Pseudomonas pyrrocinia*. Pyrrolnitrin (**28**) exhibited high antibiotic activity against dermatophytic fungi, particularly



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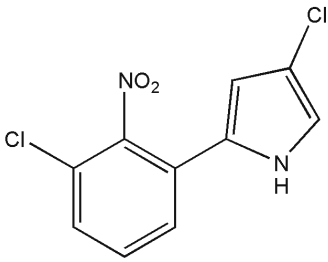

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against members of the genus *Trychophyton* and against many soil borne fungal plant pathogens like *Rhizoctonia solani* and *Fusarium sambucinum* and against foliar fungal pathogens like *Fusarium graminearum, F. culmorum* and *Pyrenophora tritici repentis*.[86] This compound was marketed in Japan under the name PYRO-ACE for the treatment of superficial dermatophytic infections.[86h] Its light sensitivity prevented the use of pyrrolnitrin (**28**) as a fungicide in plant protection. Recently, antimycobacterial activity was reported for (**28**) and related compounds.[86i] Biological activity of (**28**) at low concentrations was demonstrated to be due to the uncoupling of oxidative phosphorylation in *Neurospora crassa* and at higher concentrations due to inhibition of electron transport both in the flavin region and through cytochrome coxidase.[86*l*] However, recently it was reported that (**28**) leads to glycerol accumulation and stimulation of triacylglycerol synthesis resulting in leaky cell membranes and concomitant break down of biosynthetic activity followed by cessation of cell growth.[86m] It had been characterised as 3-chloro-4-(2-nitro-3-chlorophenyl)pyrrole. A synthesis of the antibiotic is reported.[87]

The formation of antibiotic substances of the types mentioned above gives the indication that the marine microbes are capable of producing new and unusual types of antibiotic substances than the terrestrial ones. Some of these bioactive substances would, undoubtedly, be found useful in medicine and pharmacology, while others could become of even greater interest than native product.

Serratia marcescens, a widely distributed non-pathogenic bacterium, had furnished a red coloured antibiotic named prodigiosin.[88-96] It exhibited high order of antibiotic and antifungal activities. The high toxicity of prodigiosin precluded its use as a therapeutic agent.

Studies on the marine phytoplanktons are few because of difficulty of growing the organisms and the low yield of secondary metabolites. However, several toxins related to saxitoxin are isolated from *Gonyaulax* species.[97-103] The cultured cells of the dinoflagellate *Ptychodiscus brevis*, yielded brevetoxin B, C and dihydrobrevetoxin B.[103-107] A unique feature of their structure is a chain of eleven, continuous *trans*-fused ether rings in the form of a flat ladder. *P. brevis* yielded two phosphorus containing toxins[108] GB-4 and

GB-1 which do not appear to be natural products since attempts to incorporate ^{32}P into GB-1 gave ambiquous results.[109,110] Two new polycyclic ethers, GB-5 and GB-6 closely related to okadaic acid, a toxin that was first found in sponges and later in dinoflagellate have been isolated from the cultured cells of *G. breve*.[111] The dinoflagellate *Dinophysis*, produces and transmits shellfish, toxins that are responsibe for diarrhoetic shellfish poisoning.[112] *Lyngbya majuscula* known to cause swimmer's itch has furnished several class of compounds.[113-126] Pukeleimides (A-F) showed activity against *Mycobacterium smegmatis* and *Streptococcus pyrogenes*.[113,127,128] Cyclic depsipeptide, majusculamide-C isolated from the organism inhibits the growth of fungal plant pathogen.[129] Aplysiatoxins and oscillatoxins isolated from blue-green algae *Schizothrix calcicola* and *Oscillatoria nigroviridis* possess antileukaemic activity but their high toxicity precludes their medicinal use. Cytotoxic and fungicidal nucleosides have been isolated from a variety of blue-green algae.[130] Anatoxin-a, an exogenic toxin of blue-green alga *Anabaena flosaquae*[131] is one of the most potent nicotinic receptor agonist. It is suggested that the analogues of anatoxin-a may be of clinical value for treating disorders associated with defects in cholinergic regions of the central nervous system.

Several species of green-algae of the genus *Halimeda* produce an ichthyotoxin which exhibits diverse biological activities.[132-134] It inhibits the growth of marine bacteria and fungi, cell division of fertilized sea-urchin eggs and the motility of sea-urchin sperms at 1 μg/mL. Avrainvilleol, a brominated metabolite of green algae, *Avrainvillea longicaulis* exhibits high order of antifeedant activity in reef fish and also inhibits the growth of microorganisms. The genera *Halimeda, Penicillus* and *Udotea* are found to contain highly active but unstable sesquiterpenoids and diterpenoids. Some of these diterpenoids exhibit cytotoxic and antimicrobial activities.[135,136] Prenylated aromatics with small side chains are relatively common in brown algae.[137] Several highly unsaturated C_{11} hydrocarbons are isolated from *Dictyopteris plagiogramma* and *D. australis*.[138,139] The function of these hydrocarbons have been studied in detail.[140,141] It has been observed that the sperm cells aggregate around the female gametes of brown algae which exude C_{11} hydrocarbons that attract the former and cause them to remain in the excited state in the vicinity of the latter. The sex attractants that have been identified are: ectocarpene from *Ectocarpus siliculosus*,[142] fucoserratene from *Fucus serratus*, multifidene from *Culteria multifida*, n-butyl-cyclohepta-2,5-diene from *Dictyota dichotoma*, desmarestene from *Desmarestia viridis* and tinavarrene from *Ascophyllum nodosum*. Tracing the origin of arsenic in lobsters and in fish, it has been found that the brown algae *Ecklonia radiata* concentrates arsenic in the form of arseno-sugars.[143,1444] Hydroxydictyodial from *Dictyota spinulosa* inhibits feeding in the omnivorous fish *Tilapia mossambica*.[145] Three ichthyotoxic and phytotoxic diterpenes are isolated from *Dilophus fasciola*.[146,147] Several diterpenes from *Dictyota* species exhibit significant cytotoxicity.[147] Two phlorotannins from *Ecklonia kurome* exhibit