Ten years of marine natural products research at Rhodes University

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Marine invertebrates, algae and microorganisms produce a plethora of structurally unique and biologically active secondary metabolites. The ecological roles of these natural products, although not completely understood, range from chemical defence against predation to intra-specific cues for larval settlement. Surprisingly, a number of these metabolites have also shown potential as new medicines for the treatment of a variety of diseases including cancer. The natural products chemistry of southern Africa's unique marine flora and fauna is relatively unknown and this review provides an overview of the contribution made by the marine natural products research group at Rhodes University to the isolation, identification and synthesis of biologically active natural products from southern African marine microorganisms, algae, sponges, ascidians, soft corals and molluscs.

Introduction

The coastline of southern Africa, stretching approximately 3000 km from southern Namibia in the west to southern Mozambique in the east, can be divided into three principal biogeographical zones: the cool temperate west coast, the warm temperate southeast coast, and the subtropical east coast.[1] Each of these zones sustains a unique diversity of endemic marine fauna and flora that can offer rich rewards for marine natural products chemists in search of novel bioactive secondary metabolites with possible medicinal properties. A South African government report published in 1972, entitled 'Drugs from the Sea,' recognized the potential for the discovery of new pharmaceuticals from southern African marine organisms.[2] The report contained the following statement: 'Very little attention has yet been paid in South Africa to the recovery of drugs from the sea. This field offers exciting and rewarding challenges to South African scientists. Once research brings down the unit cost, the sea may offer a vast potential for the production of drugs for South Africa.' Before this report appeared, South African contributions to the study of the chemistry of marine organisms largely comprised research on marine phospholipids in Cape Town[3] and on long-chain fatty acids and alcohols from marine fish oils at the National Chemical Research Laboratory in Pretoria.[4] At Rhodes University, Nunn and co-workers were sowing the seeds of the present marine natural products research programme at this institution with their structural investigations of marine algal polysaccharides.[5]

In 1977, Elsworth and Cragg at the University of Cape Town (UCT) started the first studies in South Africa of the chemistry of southern African marine invertebrates. Their initial research was in line with the then prevailing international interest in the discovery of new steroids from marine sources and involved a gas chromatography–mass spectrometry (GC–MS) study of the complex sterol profiles of two oceanic and three intertidal species of annelid worm, the ribbed mussel, *Aulacomya ater,* sea stars, *Marthasterias glacialis* and *Henrica ornate,* and the sea cucumber, *Cucumeria frauenfeldi.*[6,7] More ambitious projects included attempts to characterize toxins from the sea hare, *Notarchus leachii,*[8] and the puffer fish, *Amblyrhynchotes honckenii.*[9]

Elsworth and Cragg's attempts at the isolation and structure elucidation of marine natural products were continually hampered by their lack of access to modern high-performance liquid chromatography and high-field nuclear magnetic resonance (NMR) techniques, which at that time were accessible to emerging marine natural products research programmes overseas. A visit to UCT in 1979 by leading marine natural products chemist, George R. Pettit from of Arizona State University, provided fresh impetus and enthusiasm. Arising from Pettit's visit were the initial collections of the ubiquitous wall sponge *Spirastrella spinispirulifer,* a source of spongiostatin 4 (**1**),[10] and additional collections of the marine tube worm, *Cephalodiscus gilchristi,* which had yielded cephalostatin 1 (**2**) (Fig. 1).[11] Both compounds are undoubtedly the most significant potential anti-cancer compounds yet to be discovered from South African marine organisms.[12,13]

Despite the intense global interest in marine natural products, as an untapped source of new pharmaceuticals in the mid to late 1980s, the natural products chemistry of southern Africa's abundant marine resources was largely ignored during this period. However, since the early 1990s three research groups, led by Yoel Kashman, the late John Faulkner and ourselves, have provided significant insights into the chemistry of biologically active natural products from southern Africa's marine fauna and

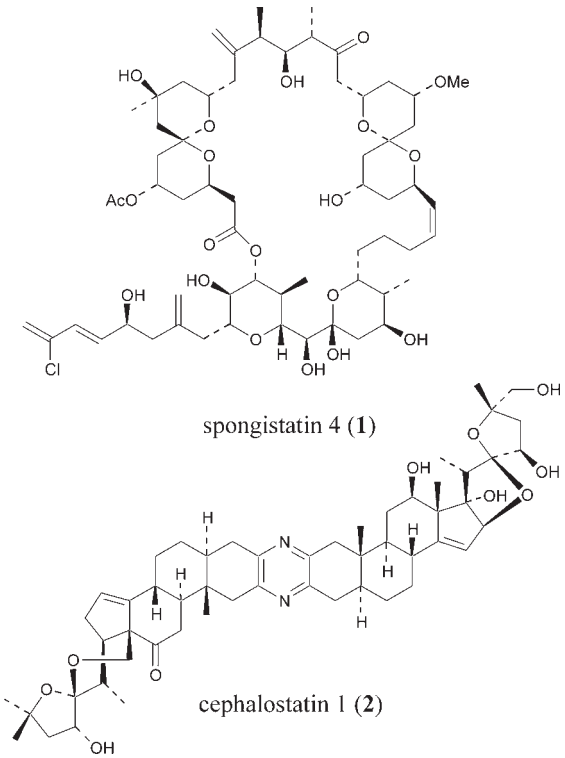

Fig. 1. Two potential anti-cancer compounds isolated from southern African marine invertebrates.

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flora. The productive collaboration between Kashman and Michael Schleyer from Durban's Oceanographic Research Institute has concentrated on the Sodwana Bay region of northern KwaZulu-Natal, while we, together with the late John Faulkner of the Scripps Institution of Oceanography, and Pat Colin of the Coral Reef Research Foundation have largely focused on our collections of marine invertebrates and algae from the temperate southeast coast of South Africa, with only two collections from the subtropical east coast (Aliwal Shoal and Ponto-do-Ouro in southern Mozambique).

Large-scale random collection of samples (*c.* 500–1000 g wet mass each of 100–150 samples per collection) of sub-tidal benthic marine organisms by SCUBA off the coast of southern Africa is expensive (R400–800 per sample) and a logistically demanding. In contrast to the more straightforward extraction of terrestrial plant material for natural products studies, the extraction of compounds from marine organisms requires the prior removal of copious amounts of water and salt, thus adding to the overall expense of the research effort. We have successfully overcome the prohibitive costs of marine collections and extractions through collaborations with the Scripps Institution of Oceanography, the former SmithKline Beecham Pharmaceuticals, the Coral Reef Research Foundation and the U.S. National Cancer Institute (NCI). Although no new potential pharmaceuticals have emerged from any of these collaborative efforts, our open access to a diversity of pharmaceutical screening programmes and ready-made extracts from all the joint collections has provided material for the plethora of natural product studies at Rhodes University reviewed here. Three groups of marine invertebrates — ascidians, soft corals and sponges — have predominated in all our large-scale collections. The phylogenetic order (ascidians, soft corals, molluscs, sponges, algae and micro-organisms) in which our results are presented reflects the chronological sequence in which we studied these groups of organisms. Representative examples of molecular structures are given in Figs 1–6, to provide an overview of the rich chemical diversity inherent in the natural products isolated from southern African marine organisms.

Natural products from marine ascidians

Marine ascidians or tunicates (Order Ascidiacea) are a dominant component of the marine benthos of the Tsitsikamma Marine Reserve and Algoa Bay.[14] Unfortunately, a global shortage of ascidian taxonomists frequently hampers the study of natural products from these organisms because publication of the structures and bioactivities of marine natural products is not possible without identification of the source material. In an effort to rectify this impasse, therefore, our collaboration with the Coral Reef Research Foundation provided an opportunity for the training of an ascidian taxonomist at the University of Port Elizabeth.[14] Ascidian extracts are the most commonly used in our in-house, anti-microbial and brine shrimp lethality assays. The latter assay is regularly used to detect cytotoxicity in terrestrial plant extracts.[15] Bioactivity-guided fractionation of an ethyl acetate extract of the marine ascidian, *Pseudodistoma* sp., collected in the Tsitsikamma Marine Reserve, revealed that the anti-microbial properties in this extract resided in a group of acyclic amino alcohols, isolated as their peracetylated derivatives, e.g. 2S-acetamido-3S-acetoxy-5E-,13-tetradecadiene (**3**).[16] A hydrophobic alkyl chain, containing polar functional groups at one end of the chain, imparts detergent-like properties to this class of compounds, making them particularly effective anti-microbial metabolites through their ability to disrupt bacterial cell walls.[16]

Natural products from marine soft corals

The benthic environment off the coast of southern Africa is varied and octocorals form a major component of southern African benthic communities found in a variety of habitats ranging from the littoral zone to the edge of the African continental shelf at a depth of 468 m.[17] The Order Alcyonacea (soft corals) is one of the six orders found in the Class Octocorallia and its members are particularly abundant along the southern African coast. Despite containing nutritionally important compounds, soft corals are rarely preyed upon by other marine organisms. A lack of predation observed in brightly coloured marine invertebrate fauna, such as soft corals, suggests that these aposematically coloured invertebrates are protecting themselves against predation *via* a natural products-based chemical defence system.

Terpenoid natural products predominate in soft corals and our initial studies of *Capnella thyrsoidea,* collected in the Tsitsikamma Marine Reserve, yielded a series of xenicane diterpenes, e.g. tsitsixenicin A (**4**) and pregnadiene sterols, e.g. 5α-pregna-1,20-dien-3-one (**5**).[18] The anti-inflammatory activity of the diterpene secondary metabolites isolated from another southern African soft coral, *Alcyonium valdivae,*[19] prompted us to investigate the anti-inflammatory activity, if any, of the tsitsixenicins. The production of reactive oxygen species, including superoxides, is implicated in the biosynthesis of prostaglandins from arachidonic acid during tissue inflammation. In a series of anti-inflammatory assays performed by Inflazyme Pharmaceuticals in Vancouver, Canada, the tsitsixenicins inhibited (>80% at a concentration of approximately 30 μM) the production of superoxide in isolated rabbit neutrophils, with only tsitsixenicin B (**6**) retaining this level of activity on tenfold dilution.[18] Similar anti-inflammatory activity was observed for three related xenicane diterpenes, e.g. 9-deacetoxy-14,15-deepoxyxeniculin (**7**) isolated from specimens of the soft coral, *Eleutherobia aurea,* collected from the Aliwal Shoal off southern KwaZulu-Natal.[20] Unfortunately, the level of anti-inflammatory activity was insufficient to warrant further development of any of the xenicane diterpenes as anti-inflammatory drugs. Interestingly, 5α-pregna-1,20-dien-3-one (**5**) stimulated superoxide production in rabbit cell neutrophils. The possible cytotoxicity of these compounds was thought to generate cell lysis and thus increase superoxide levels.[18] Pregnadiene sterols are rare in the marine environment and we were therefore surprised to isolate a further group of pregnadiene compounds including pregna-5,20-diene-3α, 7α-diol 3α-acetate (**8**) from a subsequent investigation of the endemic soft coral *Pieterfaurea unilobata,* collected by SCUBA off Port Alfred.[21]

The endemic red soft coral, *Alcyonium fauri* (Family Alcyoniidae), is one of the most conspicuous shallow water octocorals found along the southeast coast of southern Africa.[17] Specimens of this species collected near Port Alfred yielded three related sesquiterpenes including the major metabolite rietone (**9**).[22] Rietone exhibited activity (IC_{50} = 9.3 μM) in the NCI's CEM-SS cell line screen; a general screen designed to identify metabolites acting at any stage in the reproductive cycle of the human immunodeficiency virus (HIV).[22] Unfortunately, the moderate levels of activity exhibited by rietone (**9**) precluded any consideration of further pharmaceutical development of this compound.

Bioassay (brine shrimp lethality assay)-guided fractionation of specimens of *Cladiella kashmani* (Family Alcyoniidae) collected by SCUBA off Ponto-do-Ouro in southern Mozambique yielded the membrane diterpene flaccidoxide (**10**) and two closely related homologues of this compound.[23] Of the three metabolites, flaccidoxide (**10**) exhibited the greatest toxicity (LC_{50} 50 ppm) in the brine shrimp lethality assay.[23]

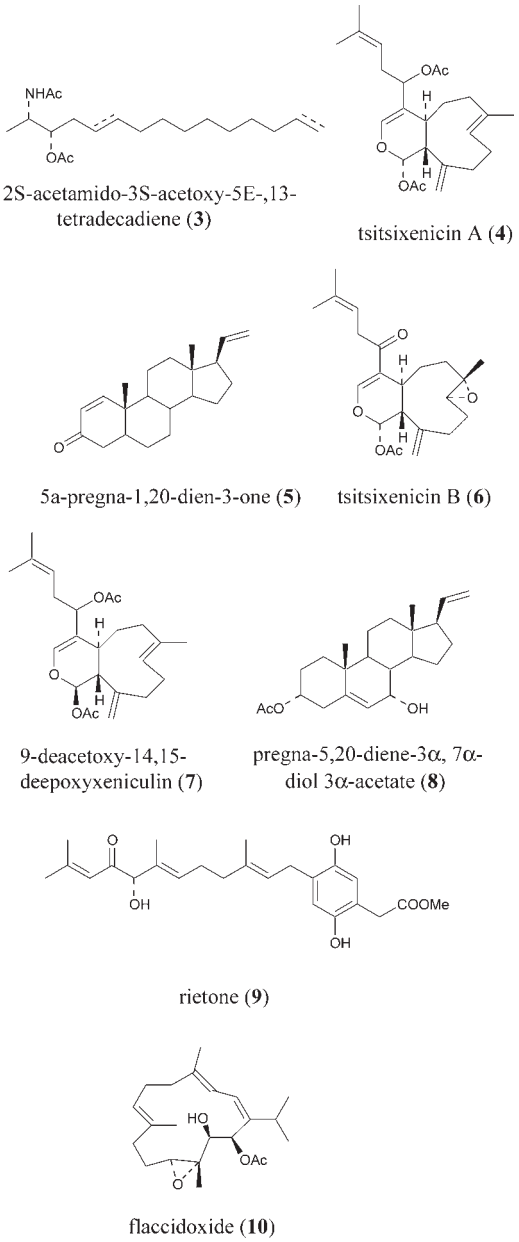

Fig. 2. Natural products isolated from southern African marine ascidians and soft corals.

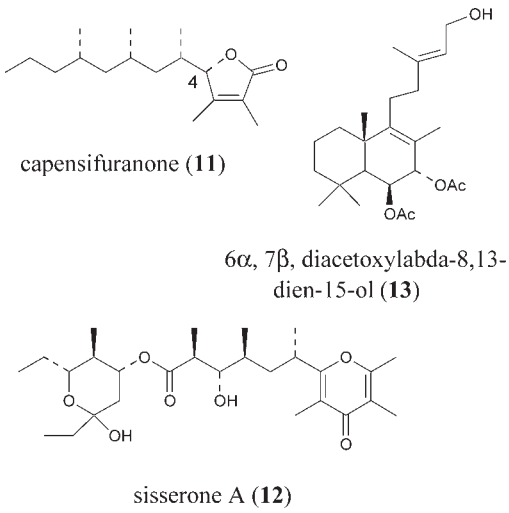

Fig. 3. Natural products isolated from southern African intertidal marine molluscs.

Natural products from marine molluscs

Our interest in the natural product chemistry of marine molluscs has focused on two genera of intertidal shelled molluscs, *Siphonaria* and *Trimusculus,* subtidal nudibranchs from the genera *Hypselodoris, Chromodoris* and *Leminda,* and sea hares of the genus *Aplysia.* Pulmonate molluscs of the genus *Siphonaria* are often referred to as 'false limpets' and nine species are known to inhabit South African shores.[24] Siphonariids are air-breathing, intertidal herbivores and are regularly submerged at high tide, thus making them susceptible to predation by both terrestrial and aquatic predators. When disturbed, siphonariids produce a white mucus from lateral pedal glands.[25] Although this mucus contains copious amounts of polypropionate metabolites, the ecological role of these secondary metabolites, or their acyclic precursors, is unknown.[26] We have examined the polypropionate constituents of two species of *Siphonaria, S. capensis* and *S. serrata.* Specimens of *S. capensis* collected from the Bushman's River mouth near Port Alfred yielded five polypropionate metabolites, e.g. capensifuranone (**11**),[27] whereas the rearranged polypropionate ester siserrone A (**12**) was isolated from specimens of *S. serrata* collected from Cape Recife near Port Elizabeth.[28] Together with Mary Garson of the University of Queensland, Australia, we proposed that the latter rearranged polypropionates are not true natural products but instead are probably artefacts generated by the chromatographic work-up of the *Siphonaria* extracts.[28] The novel polypropionate structural motifs inherent in *Siphonaria* secondary metabolites continue to provide challenging synthetic targets for organic chemists and the structure, including the (S)-stereochemistry at C-4, of capensifuranone has recently been confirmed by synthesis.[29]

Trimusculus costatus is the only known member of this genus found along the coast of southern Africa. This shelled pulmonate gastropod mollusc congregates in large groups on the underside of intertidal rocky overhangs and caves along exposed shores. From specimens of *T. costatus* collected from Cintsa West near East London, we isolated two similar labdane diterpene metabolites *e.g.* 6α, 7β, diacetoxylabda-8,13-dien-15-ol (**13**).[30] Interestingly, both diterpenes were observed to inhibit feeding of the omnivorous inter- and sub-tidal predatory fish, the spotted grunter (*Pomadasys commersonnii*), at a concentration consistent with the average amount (0.25 mg) of diterpene metabolites found in a single specimen of *T. costatus.*[30]

Nudibranchs are postulated to be products of an evolutionary trend in molluscs towards replacing a protective shell with a sequestered chemical defence system.[31] As a result, nudibranchs are generally brightly coloured as a warning to potential predators that they are unpalatable. The toxic natural products selectively sequestered by nudibranchs from their diet of toxic marine invertebrates (such as sponges and soft corals) are stored in glands lining the organism's mantle tissue[31] and can be readily extracted with acetone for studies of natural products. Consequently, an examination of the toxic metabolites found in nudibranch species occurring in a particular benthic environment provides an initial insight into the diversity of bioactive metabolites produced by other marine invertebrate fauna in that environment.

The endemic nudibranch, *Hypselodoris capensis,* is a colourful member of the family Chromodorididae and specimens of this species collected in the Tsitsikamma Marine Reserve were the source of three anti-microbial linear β-substituted sesterterpenes, e.g. (18R)-variabilin (**14**) and two ichthyotoxic sesquiterpenes, e.g nakafurans 8 (**15**).[32] A concurrent investigation of the dietary sponges preyed upon by *H. capensis* revealed that the sesterterpenes had been originally sequestered from a

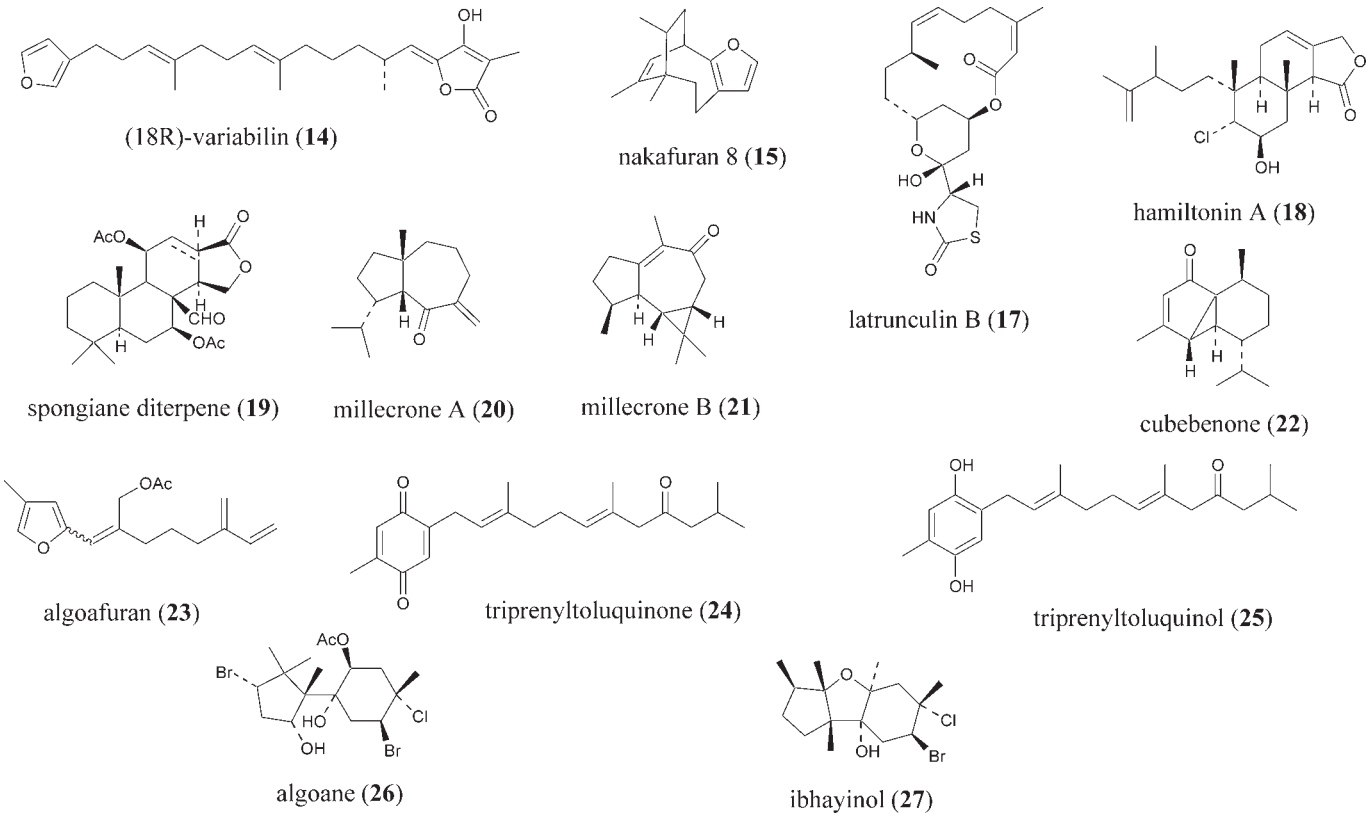

Fig. 4. Natural products isolated from southern African marine nudibranchs and sea hares.

dictyoceratid sponge (*Fasciospongia* sp.), while a *Dysidea* sponge was shown to be the source of the sesquiterpenes sequestered by the nudibranch.[33] The *Dysidea* sponge also yielded an additional sesquiterpene, tsitsikammafuran (**16**), not found in the *H. capensis* extract. The structure of tsitsikammafuran was confirmed by synthesis.[33]

Pika and Faulkner's investigation of another dorid nudibranch, *Chromodoris hamiltoni,* collected from the Aliwal Shoal yielded two toxins, latrunculins A and B (**17**), and a series of diterpenes related to hamiltonin A (**18**).[34] Latrunculin B was also present in extracts of *C. hamiltoni* collected from the reefs off southern Mozambique. The Mozambiquan specimens of *C. hamiltoni* also yielded two spongiane diterpene lactones, e.g. compound **19**.[35] No evidence of the hamiltonins was found in our investigation of extracts of the Mozambiquan specimens of *C. hamiltoni,* suggesting probable geographical variation in the organisms that make up *C. hamiltoni*'s diet in this region of the southern African coast.[35]

Leminda millecra is an attractive pink and blue nudibranch (2–3 cm long) common in Algoa Bay. From an extract made from 32 specimens of *L. millecra,* we isolated thirteen compounds including sesquiterpenes, e.g. millecrone A (**20**), millecrone B (**21**), cubebenone (**22**) and algoafuran (**23**), and triprenyltoluquinones and hydroquinones, e.g. compounds **24** and **25**.[36] Millecrone A and B (**20** and **21**) had been isolated from an earlier study by Pika and Faulkner of *L. millecra* collected off the Wild Coast.[37] Given the paucity of bioactive metabolites present in an individual nudibranchs, natural products studies of this group of organisms necessitates combining and extracting a relatively large number of nudibranchs from a given area to provide sufficient amounts (>1 mg) of individual secondary metabolites for spectroscopic identification. Such an approach obviously does not provide information about the dietary selectivity, if any, of individual nudibranchs. In our study of the dietary selectivity of *L. millecra* in Algoa Bay, we individually analysed extracts of eight nudibranchs collected by SCUBA from the 'White Sands' dive site in Algoa Bay using GC–MS techniques.[36] Cubenone (**22**) and millecrone B (**21**) were shown to be present in all eight nudibranch extracts, with the original source of these two compounds subsequently being shown to be a gorgonian (sea fan), *Leptogorgia palma,* common in Algoa Bay.[36] A GC-MS analysis of extracts of 18 soft coral and gorgonians revealed an unidentified *Alcyonium* species to be the source of millecrone A (**20**).[36]

Sea hares are also shell-less marine molluscs but, unlike nudibranchs, they prey exclusively on marine algae, concentrating bioactive metabolites sequestered from their herbivorous diets in large internal digestive glands. Although Algoa Bay is on the southern extremity of the circumtropical sea hare *Aplysia dactylomela*'s range, a prolonged period of relatively warmer water in Algoa Bay during the summer of 1998 resulted in a proliferation of this species in the Cape Recife area of the bay during this period. Our examination of the contents of the digestive gland from four specimens of *A. dactylomela* yielded a group of sesquiterpenes including algoane (**26**) and ibhayinol (**27**).[38,39] The structures of these halogenated sesquiterpenes were typical of metabolites commonly found in *Laurencia* algae, thus suggesting that this algal species forms an important component of *A. dactylomela's* diet in Algoa Bay.[38]

Natural products from marine sponges

Southern hemisphere marine sponges of the family Latrunculiidae are commonly found in the cold waters off Antarctica, New Zealand, southwestern Australia, Tasmania and South Africa.[40] Latrunculid sponges are relatively abundant on rocky reefs down to a depth of 50 m off the temperate southeastern coast of South Africa and are a rich source of bioactive alkaloid pigments.[40–43] These pigments are characterized by a central

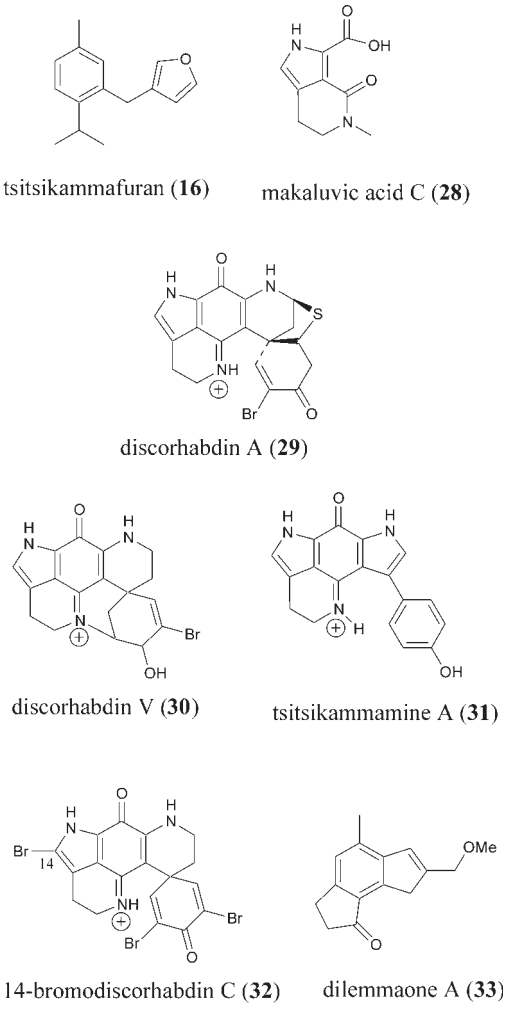

Fig. 5. Natural products isolated from southern African marine sponges.

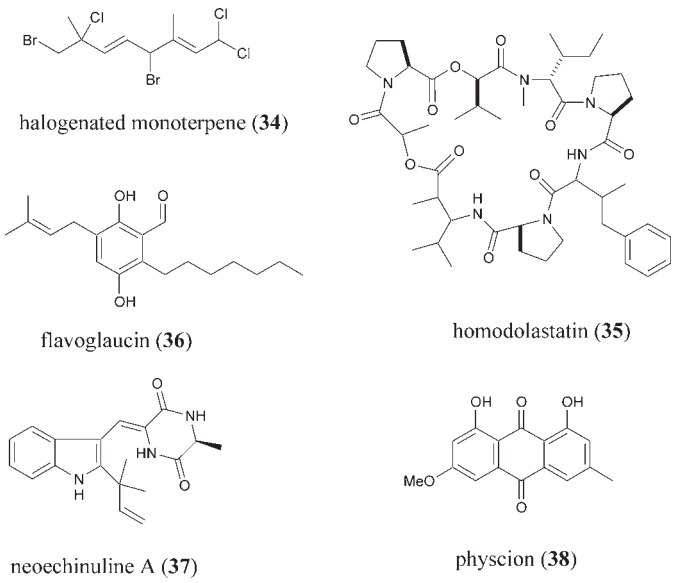

Fig. 6. Natural products isolated from southern and east African marine algae, cyanobacteria and fungi.

pyrroloiminoquinone structural motif and are often used as chemotaxonomic markers in the classification of latrunculid sponges. From our studies, over the last decade, of four species of South African latrunculid sponge we have isolated 21 pyroliminoquinone metabolites of increasing structural complexity from makaluvic acid C (**28**) to discorhabdin A (**29**), discorhabdin V (**30**) and tsitsikammamine A (**31**).[42,43] Tsitsikammamine A was the first *bis*-pyrroloiminoquinone to be isolated from a latrunculid sponge and our discovery of this compound in a new genus of latrunculid sponge, *Tsitsikamma,*[42] suggested that this compound may be a chemotaxonomic marker for this genus. Our discovery of a second member of this genus, *T. pedunculata,* in Algoa Bay, however, revealed that the *bis*-pyroloiminoquinones are confined to *T. favus* and that bromination at C-14, e.g. 14-bromodiscorhabdin C (**32**), may be a more suitable chemotaxonomic marker for this genus.[43] Pyrroloiminoquinone metabolites are renowned for their potent cytotoxicity and the suite of pyrroloimnoquinones isolated from the South African latrunculid sponges provided us with a unique opportunity to investigate the comparative cytotoxicity of this group of compounds. Interestingly, the ubiquitous discorhabdin A (**29**) proved to be the most toxic metabolite against human colon tumour cancer (HCT-116) cells (IC_{50} 0.08 μM).[43] Unfortunately, the non-selectivity of pyrroloiminoquinone cytotoxicity has precluded any further development of these compounds as anti-cancer drugs.

The screening of organic extracts obtained from 130 marine invertebrates, collected near Cape Town, for general cytotoxicity led to a bioassay-guided fractionation of three morphologically similar bright orange sponges (inadvertently mixed together in the field), resulting in the isolation of three alkaloids, e.g. dilemmaone A (**33**).[44] The dilemma surrounding the origin of the source sponge highlighted the importance of careful sorting of sponge specimens in the field and the value of the inclusion of taxonomists in expeditions to collect marine invertebrates.

Natural products from marine algae and microorganisms

The chemistry of South African marine algae and microorganisms has not been studied extensively and we have recently begun a project to explore the biomedical potential of these organisms. An investigation of the ubiquitous red alga, *Plocamium corallorhiza,* collected from Kalk Bay, near Cape Town, led to the isolation of three known and five new halogenated monoterpenes, e.g. **34**.[45] These compounds, which are related to the potent anti-cancer compound, halomon, from the red alga, *Portiera hornemannii,*[46] were screened for activity against an oesophageal cancer cell line. Unfortunately, only moderate activity was observed for these compounds, with IC_{50} values ranging from 2.01 to 13.88 μg/ml. It is interesting to note that compounds containing a geminal dichloro functional group, e.g. compound **34**, were the most active in this assay. Further studies on *P. corallorhiza* from Kenton-on-Sea on the Eastern Cape coast have shown a significant geographical variation in the metabolite profile of this marine alga.

Cyanobacteria are prolific producers of bioactive metabolites. For example, the extensively studied circumtropical cyanobacterium *Lyngbya majuscula* has yielded over 100 different secondary metabolites.[47] An examination of *Lyngbya majuscula* collected off the Kenyan coast yielded the known depsipeptide, antanapeptin A, and a new bioactive cyclic peptide homodolastatin 16 (**35**).[48] The latter compound showed modest activity against the oesophageal cancer cell lines WHCO1 (IC_{50} = 4.3 μg/ml) and WHCO6 (IC_{50} = 10.1 μg/ml) and the cervical cancer cell line, ME180 (IC_{50} = 8.3 μg/ml).

In recent years, several studies have shown that metabolites originally isolated from marine invertebrates were in fact produced by associated microorganisms.[49] Faulkner *et al.*, in an elegant study, investigated the origin of the metabolites produced by the lithistid sponge, *Theonella swinhoei.*[50] After

separation of sponge and microbial cells, they were able to detect the antifungal cyclic peptide, theopalauamide, in filamentous eubacteria, while the cytotoxic swinholide was found in a fraction containing mostly unicellular bacteria. Neither of these compounds was detected in the sponge cell fraction. This discovery has ultimately resulted in a surge of interest in marine microorganisms as a potential source of new drugs.

Using NMR to screen for secondary metabolite production, we have identified a number of fungi, associated with marine algae, that produce interesting natural products. One of these fungi, identified as a strain of *Eurotium rubrum* by sequencing the internal transcribed spacer (ITS) 1 region of ribosomal DNA, yielded three different classes of secondary metabolites: prenylated hydroquinones (e.g. flavoglaucin, **36**), diketopiperazine indole alkaloids (e.g. neoechinulin A, **37**) and the aromatic polyketides (e.g. physcion, **40**).[51]

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