The nature and role of pigments of marine invertebrates†

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Covering: 1980 to 2005

Marine animals, especially those from tropical waters, are often brilliantly coloured, and bright colouration is widespread in both sessile and non-sessile invertebrates. These spectacular natural colours are common in species inhabiting shallow waters, and appear not only in animals exposed to bright light, but also in those living in dark areas where colours are visible only with artificial illumination. Marine organisms also show variation in colour with depth and geographical location, and display great variety in colour patterning. These colour characteristics are the result of several different processes, and serve various purposes – the distribution and function of pigments seems to vary between invertebrate groups. In addition to playing an important role in how marine organisms interact, pigments may be involved in physiological processes. Although nitrogenous pigments predominate, marine organisms contain pigments belonging to all the major strutural classes of natural products, as well as some that are unique to the marine environment. This review discusses the nature and significance of such pigments, the chemical and biological processes involved, the factors responsible for and affecting bright colourations, as well as their evolution and speculation as to their function.

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† This review article is dedicated to Professor Gerald Pattenden of the University of Nottingham on the occasion of his 65th birthday.

1 Introduction

Coral reefs, often referred to as the "rainforest of the ocean", are among the most ancient, complex, and fascinating life forms that still thrive on Earth. For reasons fully understood only by nature, colour explodes across coral reefs, making them Earth's most vivid landscapes. The world's coral reefs, especially those from tropical waters, provide some of the most spectacular natural colour, and provide a pageant of great beauty. They are one of the most productive and diverse ecosystems in the world, providing habitat and food for a staggering variety of marine organisms.[1–3] Marine animals are not colourful only in the conventional sense, but also in their habits and life-styles. They have developed numerous "cunning" ways of capturing, avoiding or co-operating with their neighbours. Many animals live with corals without doing them any apparent harm. These *commensal* organisms include a variety of flatworms, polychaete worms, shrimps, crabs, brittle stars, molluscs and fish. Commensal organisms are able to live independently or with a variety of different corals. In some cases, the relationship is very specific: the commensal organism has an obligatory association with a particular coral species, or group of species, and modifies its colour, behaviour and even reproductive cycle accordingly.[2] These organisms can undergo changes in shape as part of their everyday survival strategy. Others are adept quick-change artists, bestowed with powers of mimicry to escape detection. In this almost weightless environment, the

ocean, plants and animals have the freedom to adopt shapes that would be unthinkable on land. Despite an appearance of simplicity, the tiny individuals that make up the reefs are some of the most complex in the living world. Others are primitive, such as sponges, which are metazoana 500–700 million years old.

Light and colour play an important role in how marine organisms interact. Bright colouration is widespread in both sessile and non-sessile invertebrates and these colours are common in species inhabiting shallow waters, and appear not only in animals exposed to bright light, but also in those living in dark areas where there is little perceptible colour and where colours are visible only with artificial illumination. In biology, a pigment is any material resulting in colour in plant or animal cells, and is the result of selective absorption. A chemist on the other hand, may define pigments as those compounds with absorptions in the UV-A or UV-B regions, and most importantly in the visible region. Some biological materials have so-called structural colours, which are the result of selective reflection or iridescence, usually achieved with multi-layer structures. Unlike structural colour, pigment colour is the same from all viewing angles. Butterfly wings typically contain structural colour, although many of them contain pigments as well. Because pigment colour is the result of selective absorption, there is no such thing as white pigment. A white object is simply a diffuse reflecting object, which does not contain any pigment.

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Colour results from the reflection of different wavelengths of visible light, and it cannot be detected in total darkness. In the ocean, wavelengths of light are selectively absorbed. In clear waters, red light disappears first, at a depth of about 15 m – red colouration (*e.g.* human blood) looks brown or green at depths greater than this. As depth increases, wavelengths slowly disappear until only monotonic blue remains. In turbid water, the wavelengths are absorbed more quickly.[4]

The colour characteristics of living organisms serve various purposes, and are the visual result of three different processes:[1]

(i) The metabolic formation of natural pigments or the storage of ingested pigments, both consisting of coloured molecules or chemical pigments which reflect and transmit parts of visible light;

(ii) The formation of structural colours (non-pigmented structures), which include laminations, striations, ridges, air bubbles, crystals, particles *etc.* which split light into its constituent colours by reflection, scattering, and interference;

(iii) A combination of (i) and (ii).[1]

Natural pigments are normally products of complex biosynthetic pathways involving numerous enzymes. The chemical pigments resulting from metabolic processes can be broadly classified into two classes: Those pigments directly responsible for animal colours, and the coloured secondary metabolites, which may or may not be directly involved with the visible colours of the organisms. Colouration in marine invertebrates such as the corals, sponges and squirts, may be derived from photosynthetic pigments of symbiotic zooxanthellae, cyanobacteria, or prochlorons present in the different tissues of the organism. Additionally, such colouration may be due to pigments involved in physiological processes, such as photo-protection not directly related to predator–prey interactions, due to storage of waste products or products of digestion (resulting from degradation of hormones, metabolites *etc.*, or caused by pleiotropic gene activity, cellular activity during development, or random neutral mutations at the molecular level). Colouration can be due to physiological pigments that serve in processes such as heat absorption, uptake of oxygen, digestion, and mechanical support and wound repair.[4-8] The intensity and pattern of pigmentation depend on many factors such as food supply, depth, intensity of the ambient light, and geographic location.

Colours ranging between yellow, green, blue, brown, orange, red, purple and black can be seen *in situ* in marine invertebrates (Table 1). The rich colouration of sea fans and sea feathers (Gorgonacea), blue corals (Coenothecalia), and organ-pipe corals (Stolonifera) results from carotenoids or carotenoproteins in their skeleton.[1,9-11] However, the skeletons of most corals are white, made up of calcareous matter, and their colours are due to the presence of photopigments of zooxanthellae in the polyps. A rare example of an animal that deposits a silica rather than a limestone skeleton is encountered in the sponge *Geodia mesotriaena*, which contains the silicon-containing chromoproteins geodiastatins A and B.[12] Is it possible that these metabolites play a role in the formation of these siliceous structures and the protection of the sponge surface from predators? The polyps of scleractinian corals are colourless or semi-transparent.[13] Chlorophyll-a, porphyrins (*e.g.* porphyrin C **1**), peridinin **2** and carotenoids such as **3–6** are the major pigments.[14]

When stressed (change in salinity, temperature, anthropogenic effects *etc.*), as in coral "bleaching", the organisms lose the zooxanthellae, and hence their colour. Zooxanthelate corals

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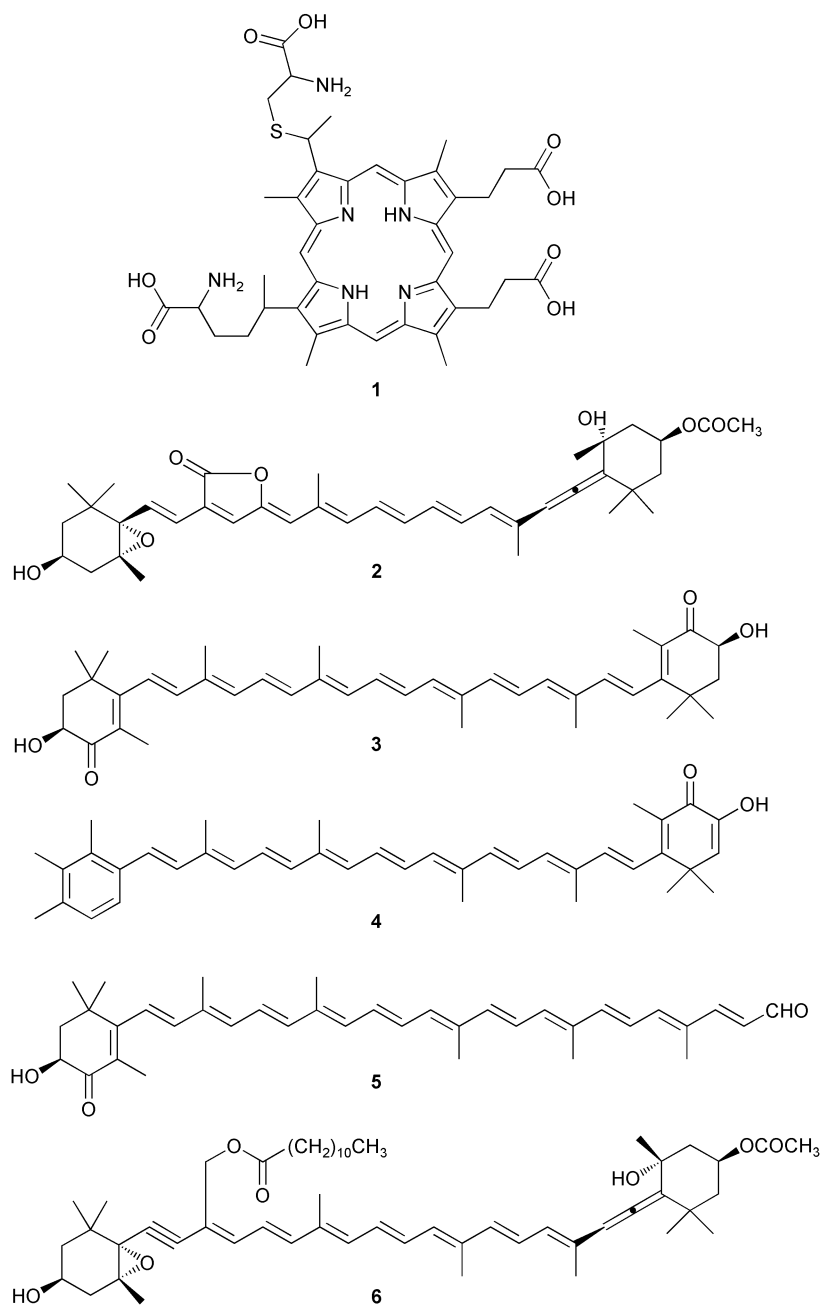
are constrained to live near the ocean surface.[3] Zooxanthellae in scleractinian corals are generally regarded as the species *Symbiodinium microadriaticum*.[15,16] Different physiological, biological, and genetic variations occur among varieties of this symbiotic alga.[16] Could the photopigments in this single species of zooxanthellae be different enough to account for the numerous colour appearances of scleractinian corals? If so, what is the pigment corresponding to each particular colouration? Or is it just the variation of the ratio between different pigments in the zooxanthellae that results in the colour differences? For example, as many as 13 distinct colour types have been recognised in the population of *Agaricia tenuifolia*.[17] If this much variation arises in the single species *Symbiodinium microadriaticum*, it would be interesting to know the nature of the variation in pigments

behind these colourful morphs. Kelmanson and Matz[14] reason that the different colours presented by some stony corals and related organisms of class Anthozoa do not originate from the composition of the pigments, but are essentially determined by a sequence of a single protein, homologous to the green fluorescent protein (GFP). They propose that the colour variation in some colonies of corals is not a true polymorphism, but rather a manifestation of phenotypic plasticity.

Marine organisms also display many examples of colour patterns.[18] The abundance and diversity of invertebrates living in "harmony", in tropical and temperate waters, suggest that the organisms have evolved protective or defence mechanisms against predation. These include behavioural (*e.g.* cryptic, nocturnal), bathymetric and morphological adaptations, physical

Table 1 The metabolites present, physical appearance and the geographical location of some marine invertebrates

Pigment[288-291]	Species[288-291]	Colour of the species[288-291]	Location[288-291]
i. Adenochromines **26a–c**	Cephalopod *Octopus vulgaris*	Yellow-green – blue – brownish-violet	Philippines, Palau, FSM
ii. Celenamide **110**	Sponge *Cliona* sp.	Rich yellow, golden-yellow and orange-yellow, and bright orange – violet-red	World-wide
iii. Celenamide **110**	Sponge *Cliona celatap*	Bright orange – violet-red	PNG, Palau
iv. Clathridine **122**	Sponge *Leucetta* sp.	White with a pale shade of violet	Antarctic, New Caledonia, Coral Sea
v. Clionamide **118**	Sponge *Cliona celata*	Bright orange – violet-red	PNG, Palau
vi. Corallistins **124a–e**	Sponge *Corallistes* sp.	Light blue	Caribbean, GBR, Japan, Mediterranean, New Caledonia, PNG
vii. Corallistine **101**	Demosponge *Corallistes* sp.	Grey to brilliant yellow	Caribbean, FSM, Japan
viii. Eudistomin **109**	Ascidian *Eudistoma* sp.	Green – brownish-violet – bright violet (mauve)	Indonesia
ix. Geodiastatins (brownish-black powders)	Sponge *Geordia mesotriaena*	Yellow – brown	Cosmopolitan
x. Isonaamidines **102e–f**	Sponge *Leucetta* sp.	Light tan, greyish-white – olive-green. Rarely pink	Coral Sea, S. California
xi. Kuanoniamine **37**	Sponge *Oceanappia* sp.	Yellow – orange – mauve – violet-brown	Atlantic, GBR, Gulf of Thailand, PNG, Palau
xii. Naamidines **102a–d**	Sponge *Leucetta* sp.	White with a pale shade of violet	Antarctic, New Caledonia, Coral Sea, Fiji
xiii. Nordercitin **113**	Sponge *Stelleta* sp.	White, grey, brownish	Caribbean, New Caledonia
xiv. Oroidin **108**	Sponge *Agelas* sp.	Brownish-orange, orange	Caribbean, Honduras
xv. Plankinidine **45**	Sponge *Plakortis* sp.	Yellow, light brown – reddish-brown – mauve	World-wide
xvi. Prodigiosin **106** (deep red)	*Pseudomonas* and marine bacteria		
xvii. Pyronaamidine **127**	Sponge *Leucetta* sp.	Yellow	Cosmopolitan
xviii. Shermilamines **40a+b**	Tunicate *Trididemnum* sp.	Ocean blue and brilliant blue	PNG
xix. Tambjamines **79a–f**	Nudibranch *Tambje morosa*	Black	Indonesia
xx. Tetrapyrrole **13**	Ascidian *Sigillina cyanea*	Pale violet – bright blue	Indonesia
xxi. Chlorin **11**	Tunicates *Trididemnum cyclops* and *T. solidum*	Brilliant green	PNG
xxii. Bonellin **123**	Euchuria worm *Bonellia viridis*	Dark green – greenish-black	GBR
xxiii. Chlorophyllone A	Mollusc (clam) *Rudiapes philippinarum*	Extremely variable. Cream to yellow with pink and brown bands	Japan, Mediterranean
xxiv. Cyclopheophorbide enol **125**	Demosponge *Darwinella oxeata*	Brilliant yellow	Black, GBR, Japan, Mediterranean, Red Sea
xxv. Discodermide **129**	Sponge *Discoderma dissoluta*	Brilliant green with bright yellow bands	Caribbean
xxvi. Didemnimides **92a–c**	Tunicate *Didemnum mambranaceum*	Green – orange – dark red – dark purple	GBR, Indonesia. Micronesia
xxvii. Fascaplysin **59** (brownish-yellow)	Sponge *Fascaplysinopsis reticulata*	Yellow to moderately violet	Guam
xxviii. Palinurin **57** (greenish-yellow)	Sponge *Ircinia spinulata*	Yellow – dark brown	GBR, FSM, Palau, Phillipines, PNG
xxix. Paragracine **119**	Anthozoan *Parazoanthus gracilis*	Green – bright red	Indonesia, PNG
xxx. Adenochrome (violet)	Cephalopod *Octopus vulgaris*	Yellow-green – blue – brownish-violet	Philippines, Palau, FSM
xxxi. Clathridine **122**	Sponge *Clathrina clathrus*	Faintly violet to "colourless"	World-wide, esp. PNG
xxxii. Eilatin **120** (blue)	Tunicate *Eudistoma* sp.	Purple	Red Sea
xxxiii. Iron-containing protein (blue)	Ascidian *Pyura stolonifera*	Brownish-violet	PNG
xxxiv. Tunichlorin **121** (blue-green)	Sea hare *Dalbella auricularia*	Variable with specks of green and brown	Caribbean
xxxv. Tunichlorin **121** (blue-green)	Tunicate *Trididemnum solidum*	Ocean blue	PNG
xxxvi. Tunichrome An-1 **32a** (yellow)	Tunicate *Trididemnum cyclops*	Brilliant green	PNG
xxxvii. Tunichrome An-2 **32b**	Ascidian *Ascida nigra*	Bright blue – violet	Indonesia
xxxviii. Tunichrome Mm-1 **32d**	Tunicate *Molgula manhattensis*	Bright blue – violet	Indonesia

FSM: Federated States of Micronesia; PNG: Papua New Guinea; GBR: Great Barrier Reef.

(*e.g.* shielding by external tissues or objects), extra-cellular structural adaptations (*e.g.* presence of protective sheaths or mucus) and chemical strategies such as the sequestration of metabolites harmful or distasteful to the predator. Theories abound as to why secondary metabolites are produced by marine organisms.[19,20] Colouration can serve in visual predator–prey relationships, and can be aposematic (warning), cryptic/homochromic (camouflage), or aid in mimicry and transparency.[21–26] This review article discusses how marine invertebrates have adapted not only to survive, but to live in harmony in a hostile environment, and the evolution, nature, role and significance of some of the pigments present.

2 Distribution

The phylum Coelenterata which includes anemones, jellyfish, hard and soft corals, hydroids, sponges (Demospongiae), followed by molluscs (Mollusca) and echinoderms (Echinodermata) have some of the most beautiful and spectacular colourations, and have some of the most unusual colour patterns amongst marine invertebrates.[2,3,5,6,9,10,27] They exhibit the widest range of pigments in the marine biosphere. Most of these colourful marine organisms are sessile and permanently attached to a substrate.[8]

Sponges show a circumpolar distribution and have adapted to a diverse array of marine habitats.[28] For example, these sessile organisms may be found beneath the Antarctic sea-ice, in tropical and temperate oceans, and (rarely) in fresh water; their size, shape and colour may also vary extensively. Some sponges are cryptic, living in secluded caves or niches, whereas others are exposed, often signalling their presence by bright and conspicuous colours. Despite their bright colours, very little is known about the chemistry of the pigments of sponges (Porifera), anemones, corals, jellyfish *etc.* (Cnidarians) and sea squirts (Ascidians). Sponges derive their colours from pigment granules located in the amebocytes[29] or from the symbionts. Pigments in cnidarians and ascidians may diffuse through the body wall or localise in spicules or skeletal materials. The spatial relationship of the pigments to hard parts, transparent areas or each other can result in the lightening or intensification of colours, or intermediate hues. Among benthic cnidarians, brightly coloured species occur in the order Hydrocorallina and most of the orders in the Anthozoa. Integumentary carotenoids in the brown and purple variants are associated with proteins of higher molecular weight and lesser solubility. Nudibranchs, also known as sea slugs, are exclusively marine, and are some of the most beautiful and diverse creatures in the ocean. They are essentially snails without shells, and their name literally means "naked gill". They have undergone complete detorsion of the visceral mass and have lost the protective shell characteristic of other gastropods.
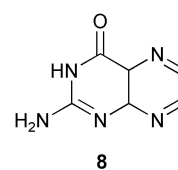
3 Historical background

Until recently, the study of marine pigments was empirical, and often the "chemistry" of the pigments was ill-defined.[1,30] The relative difficulty of collecting material, especially from deeper waters, compounded by the problem of identification, lack of available taxonomic literature and innovative analytical techniques and physical methods, may have contributed to the study of natural pigments in the earlier days being desultory and empirical. As a result, some of the early work on pigmentation should be accepted

with caution.[29] The use of fine purple dye has a long history, and has considerable antiquarian interest. The well-known and ancient "Tyrian Purple", first described by Reaumur in 1771,[31] was made from the nasty-smelling (garlic-like) fluid collected from dying muricid whelks, especially the mollusc *Murex trunculus*, and *Mitra* and *Purpura* species. The Phoenicians were the first to create an industry for its extraction, and the Babylonians used the dye extensively. Tyrian Purple was used as a dye for centuries until it was identified as 6,6′-dibromindigotin (dibromoindigo) **7** and synthesised.[32,33]

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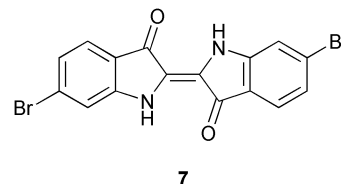
The Purple whelk is a heavy-bodied snail, often with a shell covered by frilly ridges. The exposed area of the animal is smooth and the colour can vary from white, to purple, yellow, orange and others. In the living Purple whelks, the purple exists in the form of colourless precursors which, when exposed to UV light (sunlight) are transformed into purple by sulfatases. Reviews by Kennedy,[1] Wicksten,[20,21] and Goto[35] focus on pigments and bioluminescence. Some organisms produce fluorescent pigments, and the chemicals responsible were considered to be polycyclic quinines,[36] or related to hypericin.[37–39] Fontane[40] isolated a red fluorescing pigment, a non-metallic, free porphyrin derived from chlorophyll, from the pyrolytic caecae of the black brittle star *Ophiocomina nigra* (Ophiuroidea). MacMunn[41] extracted red fluorescent "chlorophyll-like" pigments from sponges such as *Hymeniacidon sanguinea*, *Grantia seriata* and *Hircinia (Ircinia) variabilis*. They had a phaeophytin-like absorption spectrum and were thought to be derived from the chlorophyll of algal symbionts. Carotenoids and carotenoproteins were considered to be the major pigmentation of the phyla Coelenterata (Cnidaria) (corals, jellyfish, hydras, and sea anemones), Annelida (segmented worms), Echiura (unsegmented worms such as bonellies, burrow, spoon or sausage worms), Phoronidea (worm-like marine creatures), Priapuloidea Priapulid or penis worms), Sipunculoidea (peanut worms), Porifera (to which sponges belong), and Crustacea.[5–7,10,11,27,42–52] Krukenberg[53] described the lipid-soluble nature of some of the yellow and red pigments present in certain sponges. Melanins are found in molluscs in various guises and colours. Although Herring[54] reported a biliprotein in the venomous jellyfish (Cnidaria) known as the Portuguese Man of War (*Physalia physalis*), and the chromophore to be a bilatriene, Mollusca is the only phylum of marine invertebrates in which biliproteins have been detected.[1] It has been suggested that the rich colouration of Scyphozoan Medusae (jellyfish), Gorgonacea, Coenothecalia and Stolonifera results from carotenoid or chromo-proteins, chlorophyll, bilins or bilichromes, flavins, haematins, melanins, porphyrins (*e.g.* **1**), pterins (*e.g.* **8**) and quinone derivatives.[1,10,48]

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Bilins or bilichromes are metabolic products of porphyrins (*e.g.* the heme portion of haemoglobin), whose colouration can vary between yellow, green, red, and brown. They are non-metallic compounds arranged in linear, or chain, structures rather than in the cyclic configuration of porphyrins. Haematin is a breakdown product of haemoglobin. Red and orange colours appear to be the most frequent colours among Echinodermata, with a few exceptions like the bright blue starfish *Linckia laevigata* (the Pacific blue starfish) from the Great Barrier Reef and *L. miliaris* from coral reefs of the Malay archipelago.[55] Pigments include carotenoids (astaxanthin **3** is the most abundant carotenoid), carotenoproteins, flavin **9**, melanins, porphyrins, and quinones.

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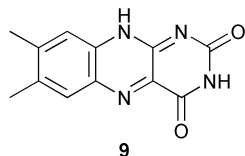
However, carotenoids are comparatively sparse in cephalopods. It is interesting to note that within the colour varieties of the plumose anemone *Metridium senile*, the white varieties contained some carotenoids, the brown varieties the smallest amounts of carotenoids (but significant amounts of melanin), and as expected, the yellow, orange and red forms the greatest quantities of carotenoids.[41,56–58] The hermit crab *Eupagurus prideauxii* is parasitised by ciliates *Polyspira* sp. and *Gymnodiodes* sp., which take up the blue carotenoproteins from the host. Carotenoids were considered to be the predominant pigments responsible for most vivid colouration.

The first reported observation of an invertebrate tetrapyrrole pigment was that of Moseley,[59] who extracted a red pigment from the anemones *Discosoma* and *Actinia*, the scyphozoans *Cassiopeia*, and from the corals *Ceratotrochus diadema* and *Fungia symmetrica*. This was later confirmed to be protoporphyrin **10**, a pigment found in the bathypelagic scyphozoans *Atolla wyvillei*.[60,61] Bonelline, a bright green pigment first isolated by Sorby[62] in 1875 from the echiuroid *Bonnellia viridis*, is a "chlorin", a dihydroporphyrin **11**.[63] Tyrosinase has been found in the tissue fluids of the black sponge *Suberites domuncula*, the orange sponge *Tethya aurantia* and the grey sponge *Cydomium (Geodia) gigas*, indicating that at least some of the dark colours (such as greys and blacks of sponges) may be due to melanin.[64] The reddish, purple and brown stripes of the jellyfish *Pelagia colorata* are due to melanins.[65]

10 **11**

R$_1$ = R$_2$ = CH=CH$_2$
R$_3$ = R$_4$ = CH$_2$CH$_2$COOH
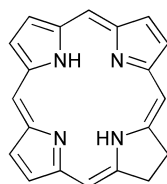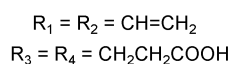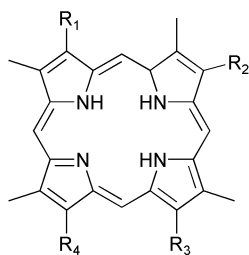
Not all coloured coral skeletons contain carotenoids. The skeleton of the brilliant blue Alcyonarian coral *Heliopora coerulea*

contains a bilichrome.[66] The crystalline violet pigment named calliactin, isolated from the anemone *Calliactis effoeta*, has a close chemical relationship to bile pigments.[67,68] The only known anthraquinone in the Annelida, a very unusual one, has been characterised from the integument of the polychaete *Halla parthenopeia*.[69] It is proposed that the deep red skeletons of the Organ-pipe coral *Tubipora musica* and the Precious coral *Corallium rubrum* are not coloured by carotenoids but by ferric hydroxide bound in the form of a complex salt with aluminium and calcium to a mucopolysaccharide. The presence of pale yellow pterin **8** (with a strong blue fluorescence) in the epidermis, and riboflavin in the dorsal integuments of Crustacea has been demonstrated.[70]

Sea hares (*Aplysia* species) secrete offensive fluids from a purple gland, and the "purple" produced by *Aplysia* consists of chromo-proteins. The heterocyclic sesquiterpene bromo-compounds aplysin **12** and aplysinol show distant relationships with the precursors of the Tyrian Purple of muricid snails.[71]

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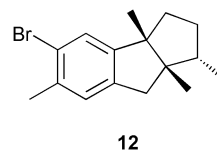
The integument of the Crown-of-thorns starfish *Acanthaster planci* contains two free naphthazarin derivatives, while two naphthoquinones have been isolated from the purple heart urchin, *Spatangus purpureus*, and naphthazarin has been found as a protein complex in the holothurian *Polyckeira rufescens*.[5,6,10,46]

4 Pigments and metabolites of marine organisms

In contrast to terrestrial plants and animals, marine invertebrates appear to have more nitrogenous pigments. Pigments include the more common and widely distributed classes, such as melanins, porphyrins, bilins, and pteridines, and exhibit groups of nitrogenous pigments (*e.g.* omnochromes) which are peculiar to them; the list can be extended to a miscellany of less common molecular types, including the indigoids recoverable from gastropod molluscs, the fluorescent zoanthoxanthins found in some colonial anthozoans, and the iron(III)-chelating adenochromes of cephalopods.[34,114] Metabolites from marine organisms represent all the major structural classes of natural products, which include alkaloids, peptides, polyketides, shikimate-derived metabolites, as well as compounds of mixed biogenesis. Metabolites arising from these biogenetic pathways have been isolated from sponges, while the majority of coelenterate metabolites are terpenoid in origin. It is hypothesised that ecological pressures have led to novel biosynthetic strategies in marine organisms. Some molluscs are dependant on dietary sources for their metabolites while some others can chemically modify the ingested compounds.[72] Another category of molluscs has the ability to biosynthesise metabolites *de novo*.[73] Echinoderms have succeeded in developing metabolic pathways for "inserting" oxygen atoms into various positions of the rings of the carotenoids and also for transporting and storing previously oxygenated molecules by conjugation with fatty acids and proteins. It is proposed that the presence of some pigments is linked to the biosynthetic activity of the symbiotic bacteria.[74–76]

This is likely, but hard to prove, as at most times the whole host/symbiont assemblage is extracted as a whole.

4.1 Carotenoids and carotenoproteins

Carotenoids, to which can be attributed colours varying from yellow to orange and red, are the most widespread class of pigments found in nature. Animals, incapable of synthesising carotenoids *de novo*, obtain their primary supplies from plants or from animals that have derived their carotenoids from ultimate plant sources, and transform them into other carotenoids. Many novel carotenoids, *e.g.* tedanin **4**, and geliodesaxanthin **5**, an apocarotenal, not encountered in terrestrial plants and animals, have been isolated from marine organisms.[20,22,77–79] For example, a series of novel marine carotenoids has been obtained from sea squirts,[80] the sea cucumbers of the order Dendrochirotida,[81] sponges,[20,29,82] and sea mat polyps (*Zoanthus* sp.).[83] Carotenoid sulfates are quite common in marine organisms. Bjornland and co-workers[84] isolated the first allenic–acetylenic carotenoid, gyroxanthin diester **6**.[78]

Non-covalent association of carotenoids with proteins is common among the marine invertebrates providing, yet again, a multitude of colours (blue, green, purple, red *etc.*). There are two major types of carotenoproteins: Type A, in which the carotenoid (chromogen) is associated stoichiometrically with a simple protein or glycoprotein, and Type B, usually less stable, in which the carotenoid is associated with a lipo (glyco) protein. Type A usually occurs at the external surface, such as the carapace of Crustacea and the skin of Echinodermata, while Type B is commonly found in the eggs, ovaries, and blood. The observed colour of a bound protein pigment depends on the binding status of the chromogen and the nature of the protein. The most frequently occurring carotenoid in the combinations is astaxanthin **3**. The extensively studied lobster pigments crustacyanin (the blue astaxanthin protein of the carapace) and ovoverdin (the green astaxanthin–lipoglycoprotein of the ovary and eggs), typify the two types of complexes. Laboratory experiments conducted with the sea urchin *Pseudocentrotus depressus* led to the conclusion that some carotenoids play an important role in biological defence. They also play a significant role as antioxidants[85] and in the reproductive processes of sea urchins[86] and holothurians.[22]

4.2 Tetrapyrroles

Following the carotenoids, the next most abundant class of pigments are the tetrapyrroles, which include haemoglobins, haematins and porphyrins.[7,50,87] The primary role of tetrapyrroles is their involvement in the processes of biological oxidation, their secondary role being their association with the pigmentation of tissues.[34] In the echinoderms, porphyrins (*e.g.* **1**) apparently manifest themselves in two ways: (i) *via* the dietary source, distributed in the hepatic caeca and integument, and (ii) as a prosthetic group in respiratory pigments. The unusual blue pigment from the nudibranch *Nembrotha kubaryana* has also been isolated from a mutant of the bacterium *Serratia marcescens*, blue marine ascidians and a blue bryozoan. Since nudibranchs are known to feed on ascidians and bryozoans, this tetrapyrrole pigment **13** present in the organism is presumed to be diet-derived.[88] Bile pigments are open-chain tetrapyrroles. The porphyrin pigments corallistins occur in high amounts in the nudibranch *Corallistes*.[89,90]


13
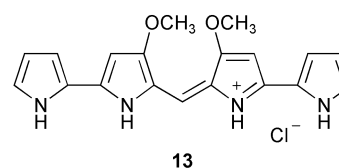
4.3 Melanins

The melanins are a broad group of pigments with greatly different structures that are responsible for dark, tan, and even yellowish or reddish pigmentations, and are derived from the aerobic oxidation of phenols. They are polymers of either or both of two monomer molecules: an indolequinone (*e.g.* 5,6-indolequinone) **14a** and a dihydroxyindole carboxylic acid (*e.g.* 5,6-dihydroxyindole-2-carboxylic acid) **14b**.
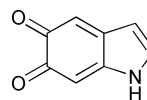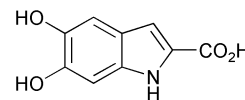
**14a**   **14b**

More broadly, melanin is any of the polyacetylene, polyaniline, or polypyrrole "blacks" or their mixed polymers. They exist in the plant, animal and protista kingdoms, where among other functions, they serve as pigments. Because melanin is an aggregate of smaller component molecules, there are a number of different types of melanins with different proportions and bonding patterns of the component molecules. Nitrogen-free melanins or allomelanins are found in higher plants, fungi and bacteria, and nitrogen-containing melanins occur widely in the animal kingdom. The latter class of pigments are subdivided into two groups: The black and brown insoluble eumelanins derived from aerobic oxidation of tyrosine in the presence of tyrosinase,[34] and the alkali-soluble phaeomelanins ranging from yellow to reddish brown, which arise by deviation of the eumelanin pathway through intervention of cysteine and/or glutathione. Eumelanins are commonly found in a variety of biological systems ranging from integuments and body fluids of various invertebrates to mammalian skin and eyes. Dark brown melanin (melanoprotein) is stored in high concentration in the ink sac of the cuttlefish *Sepia officianalis* and *Octopus bimaculatus*,[91–93] and widely distributed in the classes Echinoidea (sand dollars, heart and sea urchins), Holothuroidea (sea cucumbers), and Ophiuroidea (basket, brittle and snake stars) in the phylum Echinodermata.[94] They may be polymers arising from repeated couplings of simple bi- or polyfunctional monomeric intermediates, or may be of high molecular weight because these intermediates have coupled with large molecules, *e.g.* proteins.[91,95,96] Phaeomelanins, found in reddish hair and feathers, on the other hand, are very unusual polymers containing benzothiazole and tetrahydroisoquinoline ring systems. Common features of these pigments include high molecular weight, insolubility, and lack of well-defined physical properties and characteristics. These pigments act as UV-absorbing compounds.

4.4 Omnochromes

Omnochromes can broadly be subdivided into two groups: the yellow, orange, bright red, and brownish-violet, alkali-labile ommatins (*e.g.* xanthommatin **15**) of low molecular weight found

in crustaceans, and the dark purple sulfur-containing ommins of high molecular weight, which usually occur as a mixture of compounds and are commonly found among crustaceans and cephalopods, and situated in the chromatophores. The darkening or lightening of the Common shrimp *Crangon vulgaris* is due to the movements of ommin granules in processes involving the skin chromatophores. They are also responsible for the rapid and spectacular colour changes in the skin of cephalopods and are brought about by radial muscles attached to each pigment cell. Omnochromes occur in cells as melanosomes and are insoluble in water and neutral solvents. They arise biogenetically from tryptophan, occur associated with proteins, and are often found in animals that also synthesise melanins.[34]


15
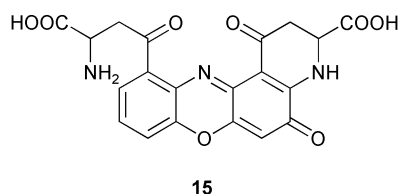
## 4.5 Phenols, pyrones, and quinones

Apart from the quinones of melanin synthesis, three kinds of variously substituted polyketide pigments, naphthoquinones, anthraquinones (*e.g.* **16**) and naphthopyrans (*e.g.* **17**) have been isolated from many families of marine organisms.[34,97] Crinoidea (feather stars) contain phenolic sulfates derived from naphthopyrones. Comaparvin **18a** and 6-methoxycomaparvin **18b** from the feather star *Comanthus parvicirrus timorensis* occur as monosulfates.[98] Ubiquinone (Coenzyme Q10, CoQ10) **19a** and ubiquinol **19b** are not conspicuous pigments, but are respiratory enzymes. The readily oxidisable hydroxyhydroquinone occurs in the sponge *Axinella polypoides*.[99]

Although naphthoquinones have been found in several classes of the marine phylum Echinodermata,[98,100] until recently, anthraquinones and naphthopyrones were confined to a single class, the Crinoids (sea lilies),[101,102] and crinemodin **20** appears to be one of the commonest crinoid pigments.[103] The sulfurous yellow colour of the Red Sea sponge *Verongia (Aplysina) aerophoba* is due to the quinoline hydroquinone uranidine **21**, which is responsible for the rapid blackening observed when the yellow sponge or the predator nudibranch is exposed to air. This colour change is due to the pH increase in the damaged cells and aerial oxidation.[29,104,105] The unusual sesquiterpenoid aminobenzoquinone 3-methylaminoavarone, **22**, is a red pigment isolated from the sponge *Dysidea avara*.[106–108] One of the most unusual quinones, halenaquinone **23**, comes from the tropical sponge *Xestospongia exigua*, which is, at least, partly responsible for its dark yellow colour.[109]

Simple bromophenols are abundant among marine organisms, and antimicrobial activity is the most common biological property observed for these metabolites. In addition, some are antibiotics, anti-cancer agents, and alarm pheromones.[110] Cadiolides A **24a** and B **24b** are found in the Indonesian ascidian *Botryllus* sp.[111] The pigment isorhodoptilometrin present in the starfish *Echinaster echinophorus* and *Henricia leviuscula* is a hydroxyanthroquinone, and the pigment is present as the water-soluble sodium salt of the 2′-*O*-sulfate. This is not unusual for crinoid pigments, which seem

16

17

18a R = H
18b R = OCH$_3$

19a

19b

20

21

22

23
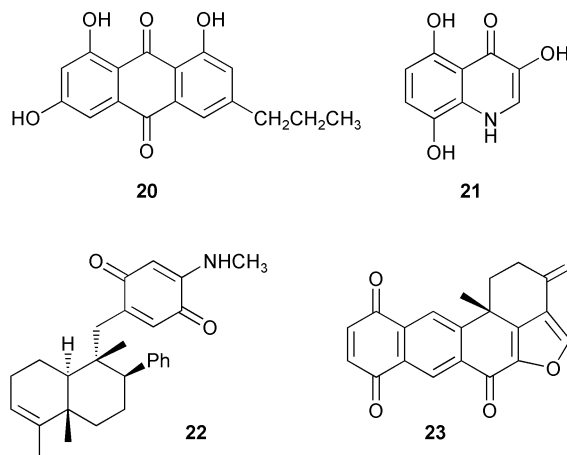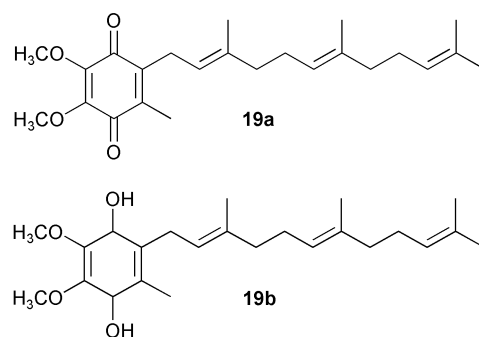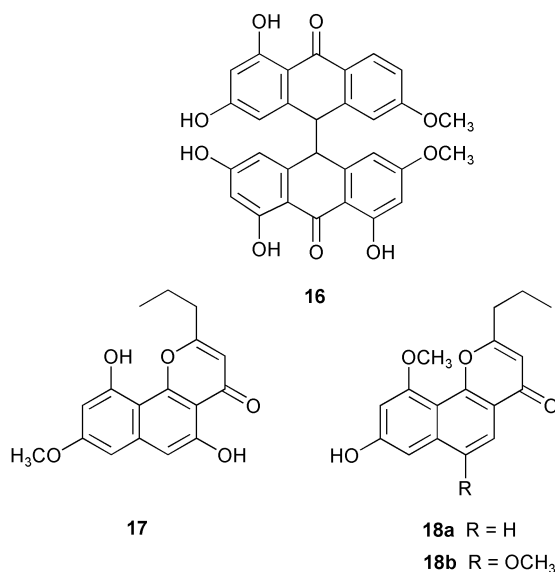
to serve as fish antifeedants, and thus has a defensive function in these starfish.[111,112] Phenanthroperylenequinone pigments, the gymnochromes, gymnochrome A, B, and C (**25a**, **25b**, **25c**) and isogymnochrome B **25d**, have been isolated from a Norfolk Ridge deep water "living fossil" crinoid, *Gymnochrinus richeri*. It is proposed that these pigments represent a conserved trait from Jurassic crinoids, while the presence of non-brominated analogues in extant land plants and ciliates may be rationalised by convergent evolution.[113]

24a R = H
24b R = Br
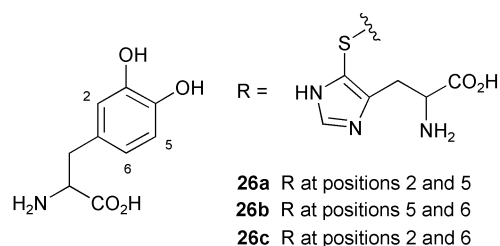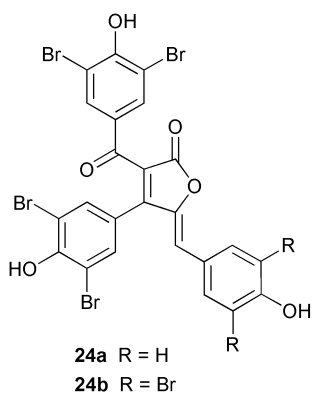
26a R at positions 2 and 5
26b R at positions 5 and 6
26c R at positions 2 and 6

27   28

29a R₁ = H   R₂ = NH₂
29b R₁ = CH₃   R₂ = NH₂
**29c** R₁ = CH₃   R₂ = N(CH₃)₂

4.6 Adenochrome and arenicochrome

Adenochrome is a non-proteinaceous pigment that occurs as garnet-red inclusions in the glandular heart tissues of the Common octopus (*Octopus vulgaris*), and in one of the largest known octopods, the giant Pacific octopus, and the Two-spot *O. bimaculatus*. This red-violet iron-sequestering pigment, which confers a characteristic purple colour to the branchial hearts, is a complex mixture of related peptides derived from glycine, and three novel isomeric catechol amino acids, adenochromines A, B, and C **26a–c**, which account for the chelate formation with Fe(III).[34,114] These catechols are obviously acting as ligands, and have the potential to bind and transport iron(III) ions. These characteristic components arise biogenetically by addition of a hitherto unknown 5-thiol-L-histidine **27** to dopaquinone formed by tyrosinase oxidation of dopa **28** (3,4-dihydroxyphenylalanine). 5-Thiol-L-histidine **27**, the parent amino acid of the yellow disulfide **29a**, and the derivatives **29b** and **29c** are found in the unfertilised eggs of various echinoderms: sea urchins (*Paracentrotus lividus*, *Arbasia lixula* and *Sphaerechinus granularis*), starfish (*Marthasterias glacialis*
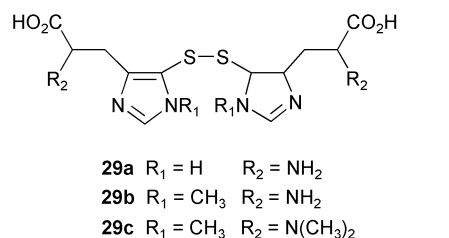
and *Astropectan aurantiacus*) and sea cucumber (*Holothuria tubulosa*).[114] The removal of iron from the pigment results in loss of the absorption maximum at 505 nm with the formation of a pale yellow substance, which is capable of binding with Fe(III) and regenerating the original pigment.[69]

The occurrence of an adenochrome-like pigment has been reported from the marine bryozoan *Bugula neritina*. The pigment is an acid–base indicator, changing from blue-purple (pH 2.4) through purple, wine-red to purple-blue (pH 11.0) and exhibits a sharp absorption band at 545 nm (aqueous solution at pH 6.8) with a shoulder around 545 nm.[114] The Common lugworm

**25a**

25b

25c

25d
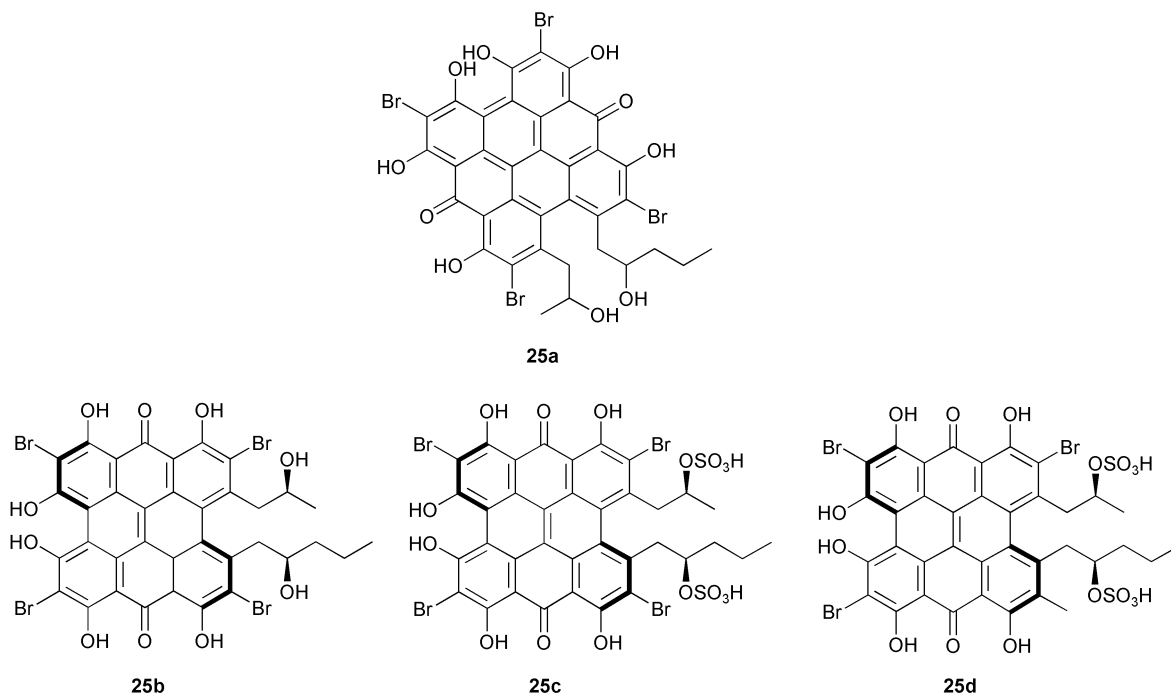
Arenicola marina, prized by anglers for bait, when found in environments with low oxygen levels, contains the pigment arenicochrome, which is a derivative of benzopyrene. The native pigment is a disulfate but the position of the sulfate has not been unambiguously determined. Hydrolysis of the disulfated pigment yields arenicochromine **30**.[114]

30
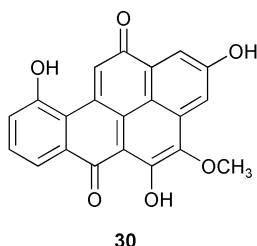
4.7 Azulenes

The strikingly brilliant colours of the tropical marine fauna include many shades of blue and purple. The molecular structure of blue marine pigments was unknown before the isolation of guaiazulene **31** from the gorgonian octacoral *Euplexaura erecta*. Subsequently, various shades of blue and purple colours in both shallow and the deep-sea gorgonians have been attributed to other guaiazulene and related sesquiterpenoid derivatives (guaianoid pigments).[115] The blue colours may also be due to other pigments such as tetrapyrroles[88] and carotenoproteins.[55]


**31**
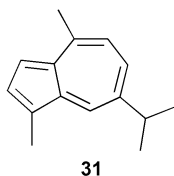
4.8 Hemovanadin and tunichromes

Certain tunicates of the Ascidiidae family (*e.g. Phallusia mammilata*) turn blue in death, due to the unusual composition of the greenish ascidian blood. This is believed to contain relatively large amounts of vanadium, sulfuric acid, and an unknown nitrogenous substance,[116] located in specific cells (vanadocytes). When separated, the green vanadocytes release the vanadium compound as a reddish brown solution (Henze solution), which on exposure to atmospheric air, turns deep blue and becomes colourless in acidic media. Various hypotheses have been proposed but little is known about the chemistry of the vanadium compound except for the observation that the green colour of ascidian blood is not due to a vanadium(III) complex. Tunichromes, *e.g.* tunichromes An-1, An-2 and An-3, **32a–c**, from *Ascidia nigra*, and tunichromes Mm-1 and Mm-2, **32d–e**, from the tunicate *Molgula manhattensis* are novel, yellow, polyphenolic tripeptides, first isolated from the vanadium-rich blood cells of the tunicate *Ascidia nigra*.[117]

4.9 Marine indoles

Except for Tyrian Purple, the isolation of marine indole alkaloids is a rather recent endeavour.[118] Tyrian Purple, Royal Purple, Purple of the Ancients and purpurin are all synonyms for an ancient dye obtained from certain gastropod molluscs, including

32a $R_1 = OH$, $R_2 = OH$
32b $R_1 = H$, $R_2 = OH$
32c $R_1 = H$, $R_2 = H$

32d R = H
32e R = i-Bu
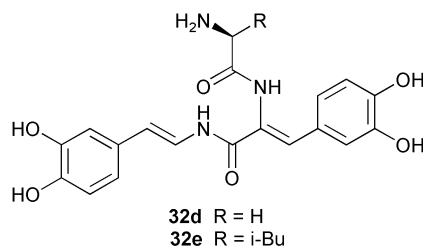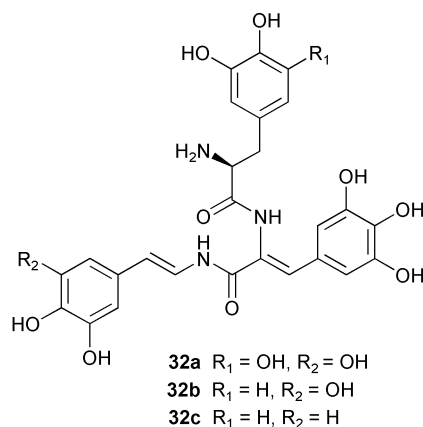
the genera *Murex* and *Purpura*. This has been identified as 6,6″-dibromoindigotin **7**; indigotin **33** was also recognised as a major component of the dye. Tyrian Purple has been isolated from an acorn worm *Ptychodera flava laysanica*, together with two other indigotin derivatives **34a** and **34b**.[114]

33

34a

34b
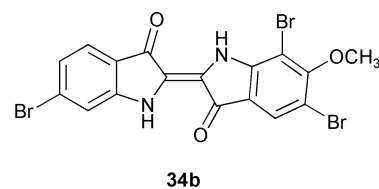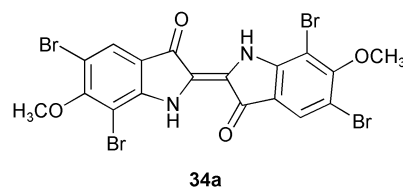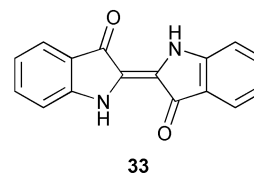
Simple brominated methylindoles are encountered in the precursors of Tyrian Purple. The precursor present in the secretion of the hypobranchial gland and in the molested gland of a number of species from the molluscan families Muricidae and Thaisidae, and the egg mass of the muricid *Dicathais orbita*, is 6-bromo-2-methylthioindoxyl-3-sulfate (tyrindoxyl sulfate) **35a**. The sulfate ester is hydrolysed by enzymatic action to produce tyrindoxyl **35b** and/or the corresponding keto tautomer. The mechanism of production of the coloured antimicrobial metabolites and Tyrian

35a **35b**
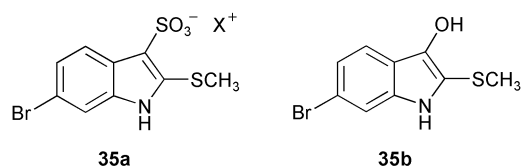
Purple in the hypobranchial glands and the egg mass of *D. orbita* is shown in Fig. 1.[114] The presence of indole metabolites, both simple and complex, is reported from several species of sponges, gorgonians, and the acorn worms. However, indole metabolites are rare among bryozoans. The odorous constituents of the hemichordate *Ptychodera flava laysanica* have been identified as chloro- and bromo-indole derivatives.[114]

4.10 Zoanthoxanthins

Most of the brilliant yellow or red colours of marine coelenterates, for example, sea anemones and stony corals, are due to carotenoids or to carotenoproteins. Among the few known exceptions are some colonial anthozoans of the order Zoanthidea (*e.g. Parazoanthus* and *Epizoaanthus* sp.) containing a novel group of yellow nitrogenous pigments, the zoanthoxanthins **36**.[119–123] Distinguishing

36 R = H or CH₃
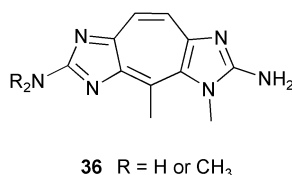
features of these pigments include their basic nature, insolubility in nonpolar solvents, stability to acids, alkalis and oxidising agents, and intense blue or yellowish green fluorescence in UV light. They were identified as diaminotetrazacyclopentazulenes at

various levels of *N*-methylation possessing either a linear or an angular skeleton.[34,124,125]

4.11 Alkaloidal pigments

Recently, a plethora of alkaloidal pigments have been isolated from a wide variety of marine organisms[74,113,126–128] (see also Sections 12–14). A large majority of metabolites have a fused tetra- or pentacyclic ring system. Interstingly, pyridoacridine alkaloids have been reported with almost equal frequency from both sponges and ascidians;[128] these organisms are often brightly coloured due to the presence of this class of alkaloids, and the colours exhibited by pyridoacridine alkaloids may vary depending on the pH. This physicochemical property of these biologically active alkaloids is correlated with the presence of at least two basic nitrogens in the aromatic ring system.[127] This class of alkaloids, which currently seem to be the largest group, is represented by the kuanoniamines, *e.g.* kuanoniamine A **37**, 2-bromoleptoclinidinone **38** (λ_{max} 355–365 nm), eilatin **39**, and shermilamines A and B **40a+b**. Many toxic pyridoacridine alkaloids have been isolated from the genus *Cystodytes*.[128]

The compounds **41a** and **41b** are commonly referred to as pyridoacridines. A review by Marshall and Barrows[128] illustrates the different structural features of the pyridoacridines, and summarises the assays used, the biological activities and structure–activity relationships for analogues of the amphimedine **42** and ascididemin **43** classes of pyridoacridines, and the mechanisms of action of their biological activities. The principal structural feature of these alkaloids is the core of a planar iminoquinone moiety which can intercalate into DNA and cleave the DNA double helix or inhibit the action of topoisomerase II.[128]

The thinly encrusting dark purple colonial ascidian, *Didemnum* sp., the mollusc *Lamellaria* sp.,[129] and the black sponge *Dendrilla*
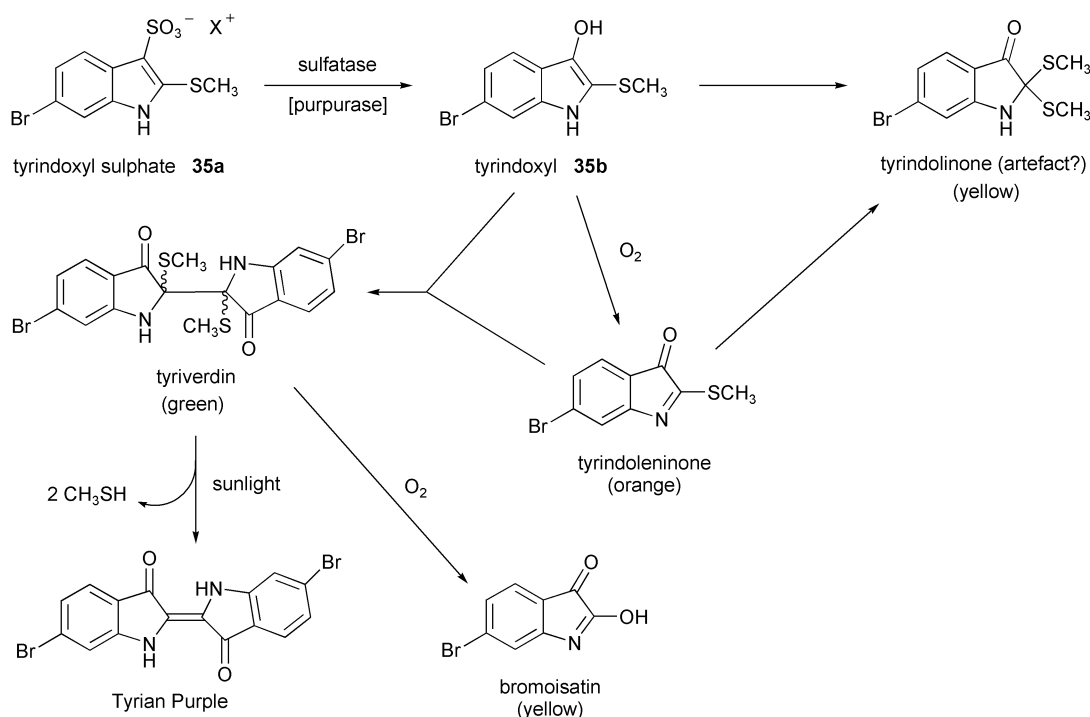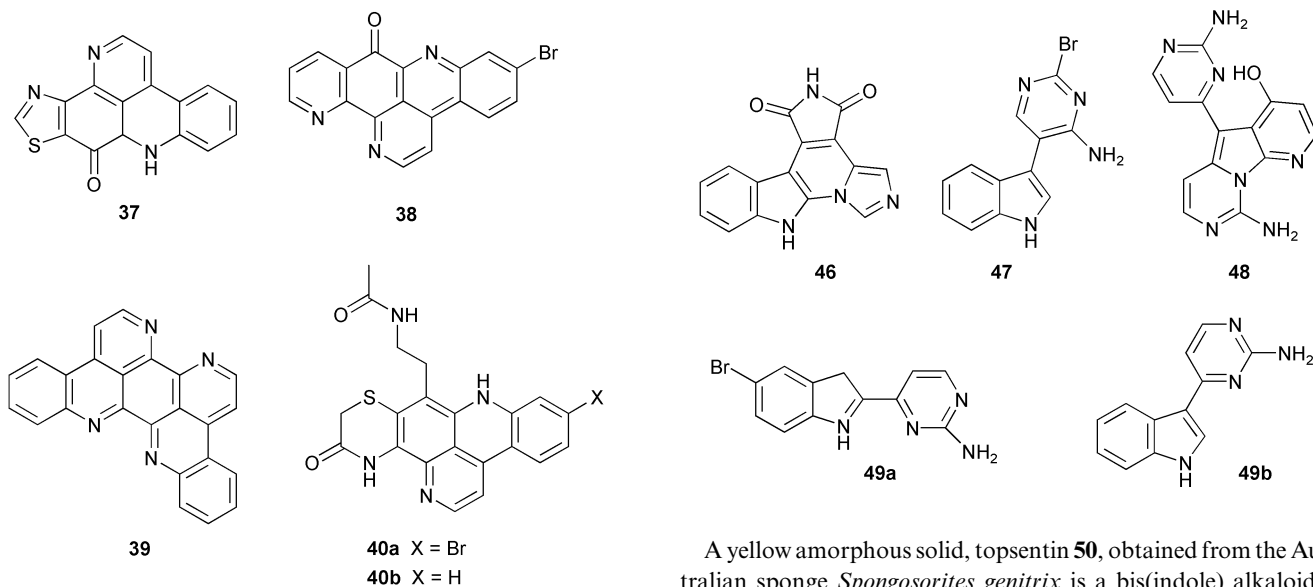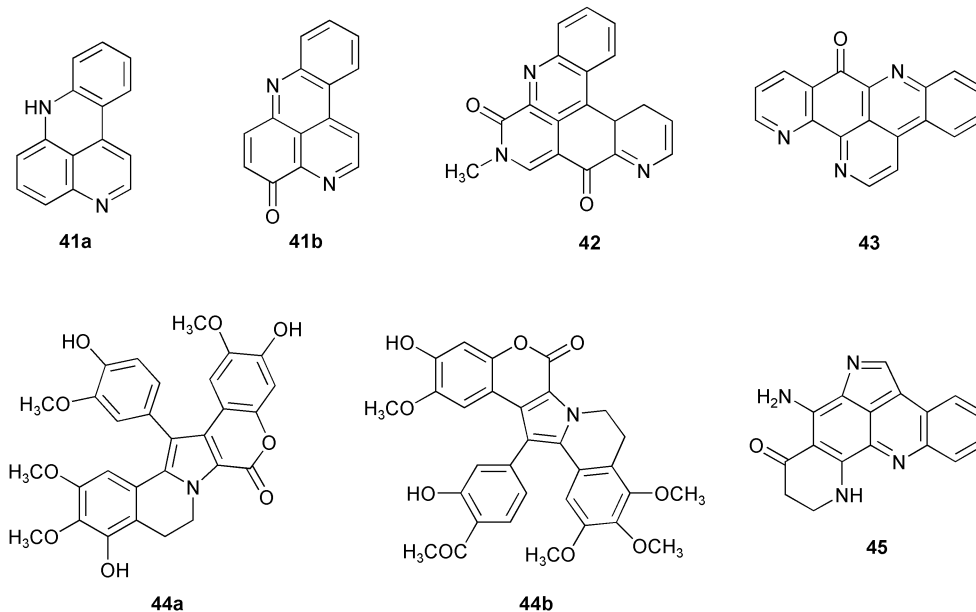

Fig. 1 The formation of Tyrian Purple from the precursor tyrindoxyl sulfate produced in the hypobranchial glands of *Dicathais orbita*.

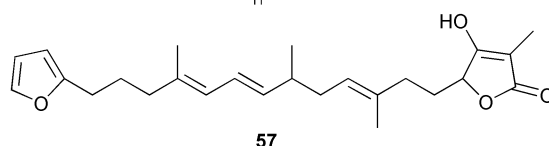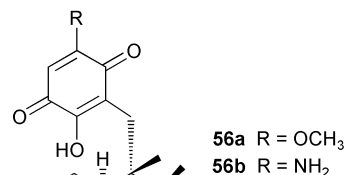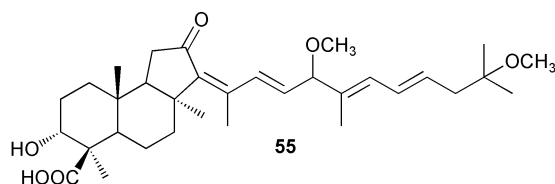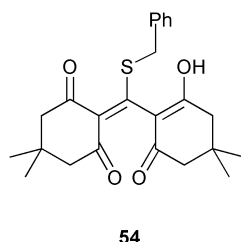This journal is © The Royal Society of Chemistry 2006

Nat. Prod. Rep., 2006, **23**, 223–255 | 233

37

38

46   **47**   **48**

39

40a X = Br
40b X = H

49a   **49b**

A yellow amorphous solid, topsentin **50**, obtained from the Australian sponge *Spongosorites genitrix* is a bis(indole) alkaloid.[76] Alkaloidal quinones have aroused considerable interest because of their antitumour properties. Microorganisms may elaborate the majority of these pigments, and this is probably true for the renierone and renieramycin groups, *e.g.* renierone and renieramycin G **51**, although they were isolated from a marine sponge. Orange sponges are conspicuous inhabitants of the Great Barrier Reef, Australia. Among them are *Phakellia*, *Agelas* and *Axinella* sp. However, red sponges are relatively rare. The bright red sponges *Kirkpatrickia varialosa*, found in the Antarctic, and *Crella spinulata*, an inhabitant of the Great Barrier Reef, are two exceptions. *Crella spinulata*, along with *Phakellia*, *Agelas* and *Axinella* sp. have yielded bright yellow metabolites with diverse chemical structures.[134] While most of them were nitrogenous compounds such as *Z*-axinohydantoin **52** and spongiacidins A and B **53a+b**, the bright yellow solid benzylthiocrellidone **54** isolated from *C. spinulata* is a novel thioether containing a dimedone moiety.[135,136]
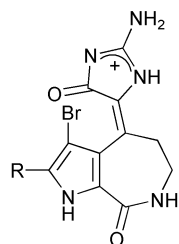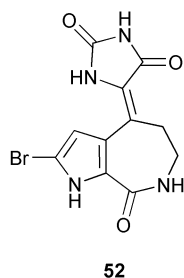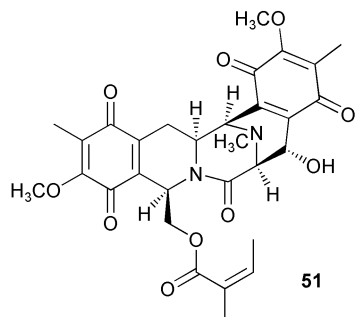
cactos[130] owe their dark colour to a series of polyaromatic alkaloids, the lamellarins, *e.g.* lamellarins E and T **44a** and **44b**. The plankinidines, *e.g.* plankinidine D **45** isolated from a *Plakortis* sp. sponge are polycyclic heteroaromatic compounds representing the first members of the pyrroloacridine class of marine alkaloids. Plankinidine D **45**, a red-orange solid (λ_{max} 328, 388, 436, 514 nm), represents the first metabolite in the pyrroloacridine family of compounds to be isolated from an ascidian.[131]

A novel alkaloid with a cyclised didemnimide carbon skeleton, **46**, is a deep purple pigment (λ_{max} 487 nm) isolated from the mangrove ascidian *Didemnum conchyliatum*.[132] The green deep-sea tunicate *Aplidium meridianum* is a source of novel indole alkaloids represented by meridianin A, psammopemmin A **47**, variolin B **48**, and isomeridianins C and G **49a+b**.[133]

41a   **41b**   **42**   **43**

44a   **44b**   **45**

50

51

52

53a R = Br
53b R = H

54

55

56a R = OCH₃
56b R = NH₂

56a R = OCH_3
56b R = NH_2

57

4.12 Miscellaneous pigments

Although sponges represent a remarkable source of new sesqui-, di-, and sesterterpenes and many triterpene saponins, only a few triterpene derivatives have been reported. Most of these are colourless metabolites, with the exception of the isomalabaricane triterpenoid **55**, a bright yellow solid isolated from a species of the sponge *Jaspis*.[137] The structurally related sesquiterpenoid quinones, ilimaquinone **56a** and 5-*epi*-smenospongine **56b**, were isolated from the sponges *Dactylospongia elegans* and *Petrosaspongia metachromia*.[138] Ilimaquinone is an amorphous powder, yellow when isolated from acidic media and violet when isolated from alkaline media. The unusual molecules palinurin **57**, isolated as a greenish-brown oil, and variabilin, a brown powder containing 25 carbon atoms and displaying at the ends a β-substituted furan ring and a tetronic acid, are characteristic of a large number of metabolites isolated from marine sponges, especially the genera *Ircinia*, *Sarcrotragus* and *Psammocinia*; they dominate the metabolite pattern of *Ircinia variabilis*. Chemical polymorphism studies reveal that, while some specimens contain either palinurin or variabilin, others contain both sesterterpenoids.[139]

Meridianins, *e.g.* meridianin A **58**, are brominated 3-(2-aminopyrimidine)indoles characterised from the green South Atlantic ascidian *Aplidium meridianum*.[133] The yellowish-brown pigment fascaplysin **59**, associated with the sponge *Fascaplysinopsis reticulata*, is a non-carotenoid pigment of the bacterium *Alteromonas* living in association with the sponge.[140] The orange anthrathiophene pigment **60** is from a bryozoan.[141] The cytotoxic

sulfur-containing pale yellow alkaloid dendrodoine **61**, isolated from the tunicate *Dendrodoa grossularia*, is unique in containing the first identified naturally occurring 1,2,4-thiadiazole ring system.[114] This compound may well equally be classified as an indole or guanidine alkaloid. A series of bromoindoles, the eudistomidines, *e.g.* eudistomidine A **62a**, isolated as a yellow gum and containing a unique oxathiazepine structural feature, has been identified from the colonial Caribbean tunicate *Eudistoma olivaceum*. Eudistomin C **62b**, isolated as yellow oil, showed very strong inhibition of *Herpes simplex* virus type 1.[114] Flavins, pterins and pteridines are rare among marine invertebrates.

58

59

60

61

62a

62b

5 Bioluminescence and biological fluorescence

Both bioluminescence and biological fluorescence phenomena, found in fireflies, glow worms and a diverse array of marine organisms, involve the emission of visible light from living species, but are distinctly separate processes.[35,142–146] Bioluminescence is the

emission of visible light either voluntarily or involuntarily, whereas fluorescence is the involuntary emission of light when an organism is subjected to ultraviolet light.

5.1 Bioluminescence

Bioluminescence, a subset of chemiluminescence, is light produced by an organism through a chemical reaction, and is the only source of light in the deep sea. Bioluminescent organisms range from bacteria and dinoflagellates to sponges, echinoderms, cephalopods (squid) and fish.[35,142–144] The bioluminescence of every organism has two very specific characteristics – frequency and amplitude. This may enable individuals of the same species to recognise each other. Two general types of bioluminescence exist. The first type, only present in fish and squid, occurs when light is produced by cultures of photophores or symbiotic luminescent bacteria that the organism maintains and manipulates through reflection or covering. Many deep-sea species, particularly crustaceans, cephalopods, and fish, use photophores to illuminate their ventral surfaces and thus disguise their silhouettes from predators viewing them from below. In some organisms the photophores are simple glandular cups, while in others, they are elaborate devices with lenses for focusing a colour filter, or an adjustable flap that serves as an on/off switch. Squid have both photophores and chromatophores within their skin and can control both the colour and the intensity of light produced. In the second type, the chemical needed for a luminescent reaction is either acquired through the food chain, or synthesised within the organism itself. Jellyfish, probably the most beautiful and spectacular of the bioluminescent marine animals, demonstrate both bioluminescence and iridescence. The chemical reaction responsible for the production of light-bursts begins with a luciferin (the photogen) and ends with a light emitter (the photagogikon), the molecule or complex that is brought into the excited state (Fig. 2). Different types of organisms use different types of luciferin molecules.
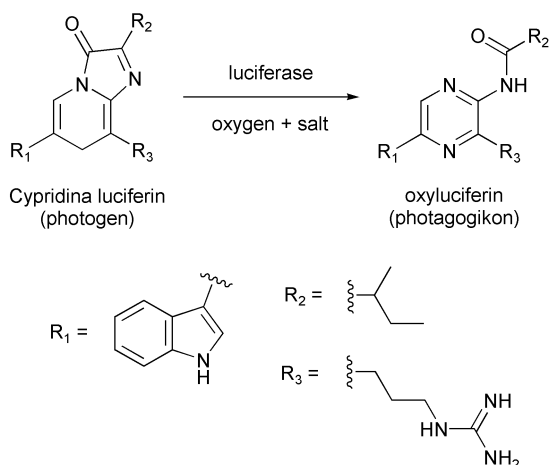
Fig. 2 Oxidative reaction of Cypridina luciferin.[145]

At times, the necessary reactants, luciferin, luciferase, salt, and oxygen, are bound together in a single unit called the photoprotein. This molecule can be triggered to produce light by another ion, generally Ca^{2+}. For example, an array of organisms such as the hydroids *Obelia* and luminescent jellyfish *Mitrocoma* use proteins, collectively known as calcium-regulated photo-

proteins, to generate light. Cypridina luciferin **63** is involved in the bioluminescence of the crustacean *Cypridina hilgendorfi*.[145]
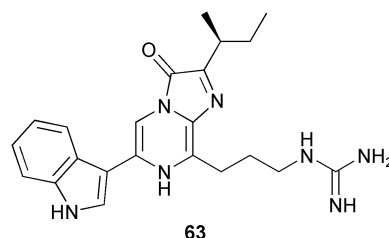
Most bioluminescence is blue for two reasons: firstly, blue light travels furthest in water, and secondly, most organisms are sensitive only to blue light. They do not have the visual pigments to absorb the longer (red light) or shorter wavelengths. However, as with every rule, exceptions exist – some cnidarians, such as the jellyfish *Aequorea*, produce green bioluminescence. Each luminescent organism has a unique flash. Factors that can vary are colour, rise, decay, and total flash times. Some organisms can emit light continuously, but most emit flashes of varying duration and brightness.[146] One of the most advantageous characteristics of bioluminescence is that of defence. The most common defensive technique is the "burglar alarm signal", seen among jellyfish. In this process, when a larger predator swims near a smaller predator feeding on its bioluminescent prey, the prey uses its bioluminescence to attract the larger predator, resulting in the fleeing of the smaller predator![142–144] Another very important characteristic that is enhanced by the luminescent ability of marine animals is that of mating behaviour. Some marine species have disperse populations, limiting the net reproduction that can occur. Bioluminescence is one mechanism that would allow a species to increase its "perception distance". It is believed that in at least two species of octopods, *Japetella diaphana* and *Eledonella pygmaea*, the female emits a light of a specific wavelength to attract its mate, while avoiding the risk of being detected by a predator.[142–144] It is a fascinating phenomenon and another amazing adaptation that some marine organisms have developed to survive in their environment.[145] The protozoans *Noctilluca miliaris* and *Pyrocystis noctiluca* have great powers of phosphorescence due to the production of oxyluciferin by the reaction of luciferin with oxygen, catalysed by luciferase (Fig. 2). Coelentarazine **64** is a metabolite with a bioluminescent chromophore isolated from the jellyfish *Aequorea victoria*, an inhabitant of the cold waters of the north Pacific.
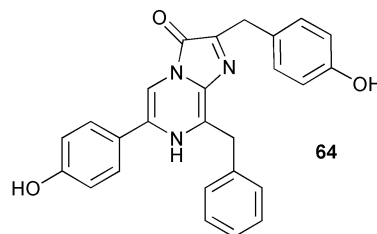
Except for a few fish and cephalopods, examples of bioluminescent lures in the sea are quite rare. In many marine organisms, including siphonophores (colonial hydrozoans), luminescence is believed to serve a defensive role. Nearly all members of this group are luminous. The siphonophore *Renna* sp., a dominant predator in the deep sea, twitches glowing lures to attract

fish, a rare phenomenon used for prey attraction among non-visual marine organisms. The lures also contain red fluorescent material that shifts the wavelength of emitted light. Though red fluorescent substances have been noted in several marine phyla, red luminescence, so far, has only been observed in the rare scaleless marine dragonflies (Stomiidae).[35] Four main uses for an organism of bioluminescence have been hypothesised. It can be used to evade predators (defense), attract prey, communicate within their species specially in the dark (*e.g.* mating behaviour, courtship camouflage, *etc.*) and advertise.[142–144,146]

5.2   Biological fluorescence

In the phenomenon of fluorescence, light is absorbed at one wavelength and re-emitted at another wavelength; therefore organisms "glow" only when light of a particular wavelength strikes them. Fluorescence is fairly widespread in reef-building corals and sea anemones, and they display a remarkable variety of fluorescent colours. The jellyfish *Aequorea* also produces green bioluminescence from small photo-organs located on its umbrella. The process is initiated by calcium ions released by the organism, which bind to a protein named aequorin, which then emits blue light. The blue light is absorbed by another protein, Green fluorescent protein (GFP), which in turn gives off green light. GFP, yellow under typical "room light", absorbs ultraviolet light from the sun, and then emits it at as lower-energy green light.[145]

Fluorescent pigments may act as natural sunscreens, aid in photosynthesis or serve as warning colouration.[146] Some marine organisms use colour-based visual signals to attract mates, warn rivals, or confuse predators. In an aquatic environment, colour-based visual signals may be unreliable because of variations in the optical properties of water, such as water opacity and the angle of incident light. It has been proposed that fluorescence may enhance colour in aquatic organisms. Some corals exhibit striking fluorescent colouring, while some squids have fluorescent markings.[146] The monogamous male Mantis shrimp, *Lysiosquillina glabriuscula*, found in the western Atlantic ocean, when confronting predators or rivals, raises its head and thorax, spreading its appendages so they appear larger. This posture also accentuates the yellow fluorescent patches on the organism. The wavelength of this fluorescence (524 nm) transmits well through sea-water.[147]

6   Colour related to biological processes

Colonial ascidians display multiple body colours. Black, orange-red, violet, white, and yellow colours are due to pigmented blood cells that exist particularly around a branchial siphon or on an atrial languet of individual zooids. These pigment cells are distributed in the mesenchymal space or vascular lumen, and many of them are loosely bound to the epithelium. The pigment cells have a spherical shape with no dendrites, and contain many types of granules; the pigmentary tissues contain carotenoids, pteridine, and purines. The main component of black and violet pigments is thought to be a melanin-like substance yet to be identified. Cephalopods, skilled in the art of colour change, which is used for camouflage or to startle and warn predators, have special chromatophores connected to the nervous system in their skin. They are able to vary their colour and even create changing patterns by controlling the size of the cells by muscular contractions.

Haminea navicula, a sand-ploughing and -digging Mediterranean and Atlantic cephalaspidean snail, is able to change the colour pattern of its body to adapt it to the surrounding sea-floor. Colour changes occur by pigment migration in ramified epithelial and subepithelial cells, by extension and contraction of melanophores. Within a few hours, some areas of the body can change from being totally "black" to nearly white.[148] The erythrophores and melanophores of the decapod *Uca uruguayensis* show a circadian rhythm of pigment translocation. The predominant erythrophores determine the typical colouration of the species, which is red during the day and beige at night. After eyestalk removal, the animal becomes permanently red in colour.

The gastropod mollusc, the ormer, *Haliotis tuberculata*, has the appearance of typical abalones, characterised by a flattened, ear-shaped shell, covering and protecting the soft body parts, and with a large muscular foot which fills the shell opening. The epithelium where there is a high concentration of toxins is denominated as the "foot side epithelium" and the epithelium of the foot in contact with the substrate is denominated as the "foot sole epithelium". Melanin and photosynthetic pigment granules in the epidermal cells of the foot side epithelium give this skin its appearance and colour, and the paralytic shellfish toxins (PSTs – saxitoxins and their derivatives) are located in specific cells of the foot side epithelium, which are distinct from the epidermal cells. Conversely, the epidermal cells of the foot sole epithelium do not have pigment granules, and PSTs have not been detected in the foot sole epithelium.[149]

The anterior sensory vesicle of ascidian larvae is a single large vesicle in which lie two types of pigment cells, the anterior and posterior pigment cells, called the otolith and the ocellus respectively. Experimental results suggest that the otolith is used for gravity detection, and that the ocellus is used for photoprotection.[150] It is suggested that the pigment cells in sea urchins (Echinoidea) embryos play a role in gastrulation.[151]

7   Colour related to genetics

The consistent range of colours and patterns in certain organisms, even in polymorphic species, is probably under genetic control, and these colour patterns are not affected by environmental conditions or the dietary source. Some sessile marine organisms show great variation in colour in the same habitat and locality.[18,152–154] Some ascidians show bands of silver derived from purine pigments, present in granules in blood cells, which are under genetic control.[5,6,8] Colour patterns may also be affected by processes occurring during the development stage or may be due to random neutral mutations at the molecular level.[18,155] One-dimensional morphogenetic programmes usually control colour patterns on mollusc shells. In adult cypraeid gastropods (cowries), by comparison, colour patterns are two-dimensional in morphogenesis and three-dimensional in structure. Visible patterns usually result from the uneven thickness of a pigmented layer, rather than from a spatially uneven concentration of pigment.[156]

8   Photo-protective pigments

The biologically important solar UV-A and UV-B radiations have deleterious effects (especially UV-B, due to its actinic nature) that are manifested genetically, physiologically and photosynthetically,

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and are potentially damaging to many forms of life, including marine organisms. In addition to increases in temperature, UV radiation has been implicated in the phenomenon of coral "bleaching". High levels of solar radiation affect photosynthesis of the symbionts, coral calcification, and survival of the organisms. However, the abundance of diversity of invertebrates in tropical waters suggests that protective or defence adaptations have evolved to combat the harmful effects of UV radiation. Amongst the diversity of methods used by marine organisms to reduce damage caused by UV radiation, the synthesis of UV-screening pigments is almost ubiquitous. Across diverse taxonomic groups of marine organisms, there are several classes of compounds that absorb UV light and act as putative sunscreens. Mycosporine-like amino acids (MAAs), with UV absorptions in the region 310–360 nm, are believed to be such a class of compounds.[23,157–160] Mycosporines can be considered to be Schiff bases (enaminoketones), and derivatives of aminocyclohexenone **65a** or aminocyclohexenimine **65b** rings. Being "colourless" compounds, some may not consider MAAs as pigments. Nageli and Schwenderer[161] first described the brown colouration of some cyanobacteria, particularly in cyanobacterial mats. This colouration is now ascribed to scytonemin **66**, which is presumed to have a dedicated photo-protective as well as an antioxidant role. Scytonemin **66**, with a bimodal UV absorption at 252 and 370 nm (*in vivo*), is a lipid-soluble phenolic and indolic derivative produced in the sheaths of cyanobacteria.[157,162–164]

65a

65b R = alkyl

66
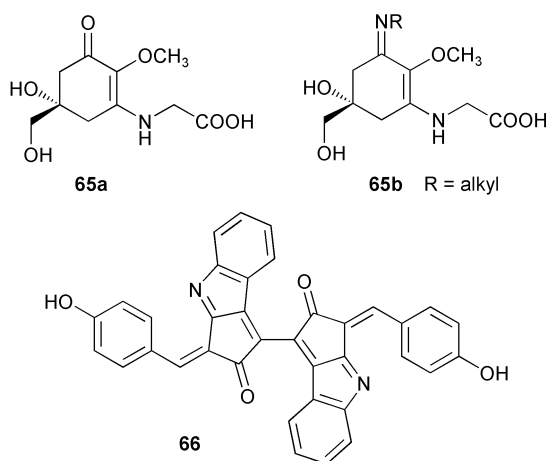
The UV-protective role of melanins is well established. Many marine species possess the tyrosinase-mediated pathway to synthesise melanin, and in fact it occurs in a wide range of marine taxa. They can act as both optical filters and antioxidants.[165] However, very little is known about the mechanisms, their efficiency or their UV-protective role in aquatic taxa. Other compounds such as phlorotannins, sporopollenin (a polymer that constitutes the outer walls of spores and pollen grains), coumarins, tridentatols (*e.g.* **67**) and polyphenolics have been implicated as UV protectants.[159] Carotenoids are photopigments that have two major functions. Firstly, they are supplemental photosynthetic pigments that absorb light energy in the blue region. Secondly, they quench oxygen free-radicals, thus acting indirectly as photo-protective pigments.[166–168] Our understanding of the range of UV-screening compounds in marine organisms is still in its infancy. The wide distribution of compounds such as MAAs suggests that a large number of compounds falling broadly into well-defined groups may be found in the future. Some may be represented in only

a few species. The oroidin alkaloids, which are various shades of yellow and are represented by *Z*-axinohydantoin **52**, sporangiacidins B and D **53a**+**b**,[169] debromohymenialdisine **68**,[134,170] thio-compounds such as the tridentatols (*e.g.* **67** from a marine hydroid) and benzylthiocrellidone **54** from the sponge *Crella spinulata*, all absorbers of both UV-A and UV-B radiation, are a few such examples.[134–136,158,171] Many of these secondary metabolites probably have multiple functions. For example, tridentatols serve as allelopathic agents, antioxidants, and sunscreens.[159,171–173]

67

68
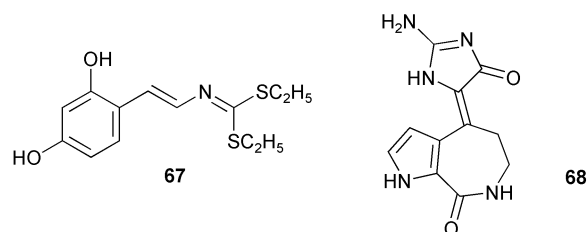
9 Environmental influence on colour

Evidence for environmental effects on colouration in marine invertebrates is scarce. The colour of invertebrates varies according to depth, water temperature, food source, currents, geographic location, light attenuation and sedimentation, *etc.*[18,174] For example, the carotenoid content in certain sea anemones decreased with depth.[50] Species with photo-protective pigments appear "bleached" in darker and deeper locations and some sponges are less brilliant (*i.e.*, they have reduced pigmentation) in shaded locations than in well-lit areas.[175,176] Photo-adaptation to decreasing flux generally results in a marked change in concentration of photosynthetic pigments contained within the zooxanthellae and shows three common trends. The first involves an increase in chlorophyll-a and the accessory pigments chlorophyll-c and peridinin with decreasing flux. The second is the relatively high concentration of compounds with UV absorption over the range of 286–340 nm in corals from shallow reefs. The third trend is the frequently higher concentrations of β-carotene and yellow xanthophylls in shallow-water corals.[174,177]

Colonies of the colonial ascidian–cyanophyte symbiosis *Trididemnum solidum* show morphological variation relative to the light regime in which they live. Colonies growing in full sunlight are white, thicker, and heavily calcified, while the shaded colonies are purple, thinner, and have a larger amount of phycoerythrin (an accessory photosynthetic pigment with an absorption in the green region of the spectrum) relative to phycocyanin (a pigment that absorbs red light) in their symbiotic algae. The purple colouration of low-light colonies appears to be due primarily to the phycobilin pigment of the algae.[178] This results in different colourations of algae growing under different lighting conditions. *T. solidum* from the Western Atlantic region is ocean-blue in appearance while *T. cyclops*, present in the tropical Pacific region (Papua New Guinea) is brilliant green. Perhaps the most remarkable adaptation shown by a coral to reduced radiant flux and different spectral quality is that of the deep-water stony coral *Leptoseris fragilis*, which has a turquoise or green fluorescence and lives at depths of 100–150 m in the Red Sea. The efficient use of the available solar energy is affected not only by the pigment changes in the symbiotic algae, but also by the fluorescent pigments sited within the coral

host tissues. Short-wavelength radiation, which would otherwise not be absorbed by algal pigments, is absorbed by the coral pigments and fluoresced into longer wavelengths for harvesting by the zooxanthellae. Such adaptation enables *L. fragilis* to colonise a habitat which is barren of all other symbiotic coral species. Furthermore, this mechanism is potentially present to some degree in all corals at all depths.[179–182]

Different species of fiddler crabs occupying a variety of intertidal niches along the Texas coast have adapted to a specific array of physical factors in the environment, and some aspects of their adaptations are reflected in body colour. Interspecific differences in morphological colourations are correlated with camouflage and substrate characteristics. Intraspecific colour variation is expressed through neurosecretion-mediated physiological change in cellular pigment distribution. Adaptation to a dark- or light-coloured background reveals different "secondary" chromo-motor capabilities for each species. In addition, pigments in melanophores, leucophores and erythrophores exhibit circadian rhythms of dispersion and aggregation.[183] Colouration is often uniform across vast geographic ranges, thus indicating a degree of genetic control over the development and expression of colour patterns. There are also species capable of matching a variety of host colour patterns, which indicates a degree of environmental influence.[184] However, this raises the question of how host colouration is perceived and translated by newly settled juveniles.

Sodium cyanide, widely used for the capture of reef fish throughout Southeast Asia, causes extensive fish mortality. The exposure of corals to cyanide caused deformation of the symbiotic zooxanthellae along with pigment loss, eventually resulting in coral mortality.[185]

10 Chromatophores

Chromatophores or pigment cells are colour-changing cells that are directly innervated by central motor neurons and used most notably by chameleons as well as cephalopods such as squid, octopuses and cuttlefish. This behaviour is generated primarily by chromatophores, which are composed of a single highly developed chromatophore cell and numerous muscle, nerve, glial and sheath cells. These cells are contractile and contain vesicles that contain three different liquid pigments, and the three types of chromatophore are characterised by the colour they carry. Erythrophores contain reddish pigments, such as carotenoids and pteridines. Melanophores contain black or brown pigments, the melanins. Xanthophores produce yellow pigments in the form of carotenoids. Leucophores and iridophores, unlike the above types of chromatophores, are unable to produce pigments and therefore are colourless. Cells carrying more than one pigment are called compound chromatophores, and various hues are made possible by the combination of different layers of chromatophores. Chromatophores are mainly located beneath the scales or in the dermis, but are sometimes seen superficially in the epidermis. They are responsible for the quick adaptation in colour required for camouflaging.

The colours generated by the cells can be broken down into two categories: biochromes and schematochromes. Colours due to microscopic, natural pigments that produce colours chemically are called biochromes. Their chemical composition is designed to absorb some colours of light and reflect others. The projected colour is a combination of all the visible wavelengths of light that are reflected by the pigment. Conversely, those colours produced by light reflections from a colourless surface and refractions by the tissues are named structural colours or schematochromes. These structures act like prisms by refracting and scattering visible light, and ultimately reflect a certain combination of colours.

Colour changes involving biochromes and some changes involving schematochromes depend on the movement of pigments within the colour cell. These short-term, rapid changes are called physiological colour changes, a phenomenon mostly found in fish and usually resulting from the animal's response to an alteration in the surroundings. On the other hand, morphological colour changes are long-term changes, occur during different life stages of the animal, and are due to increases or decreases in the number of chromatophores and a general alteration of pigmentation. Most fish that appear to have green colouration on their scales actually have a layer with yellow pigment and another layer on top that scatters light and reflects a blue colour. To change their colour, the cells distort their form or size, stretching or contracting their outer covering, and thus changing their transparency or opacity. Body patterning in unshelled cephalopod molluscs is the ability of these animals to create complex patterns on their skin. Pigment granules lie within the chromatophore cell in an intracellular sac, the cytoelastic sacculus, which has elastic walls. The contraction and retraction of the muscle cells causes the sacculus to change its shape, bringing about a colour change in the organism.[186] The tremendous capacity of the chromatophore system in cephalopod skin to change its colour across space and time has allowed these animals to produce complex visual displays to conspecific, and heterospecific targets.[187] The chromatophores in crustaceans, which have fixed shape, bring about colour change through the dispersal (darkening) or aggregation (lightening) of granules within the cells. The aggregation and dispersal of colour pigments in chromatophores seem to be under both hormonal and neural control.

11 Adaptive colouration

Adaptive colour patterns and variation in animals were at the centre of early conceptual advances in evolutionary biology, and were prominent in early thinking about evolution.[188] Much of the literature on adaptive colouration and colour patterns has been particularly concerned with predator–prey relationships. Part of the colour pattern is inherited from a common ancestor and part is due to convergent evolution, which can result from Batesian or Mullerian mimicry.

11.1 Aposematism

The term *aposematism* (literally, "away signal") is commonly used as a synonym for warning colouration. It is the combination of a conspicuous signal and an unprofitable trait in a prey species. Aposematism, a widespread trait in invertebrate taxa, and a well-documented phenomenon in nature, is the association, in a prey organism, of the presence of a warning signal with unprofitability to predators. Aposematism works by advertising to potential predators, rather than by hiding or escaping from them. Aposmetism has benefits for predator and prey. Efficient interactions between predator and prey in this context allow each to pursue other activities such as mating, care for offsping

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and feeding. It is one of the oldest phenomena in evolutionary biology and still a major puzzle to biologists. According to some evolutionary biologists it is paradoxical that an association between conspicuous signals and unprofitability could evolve. It would be difficult for conspicuous signals to evolve in prey with unprofitable traits because predators will quickly sample the prey and kill it, eliminating any genes for the conspicuous signal from the population. An alternative scenario is for unprofitability to evolve in prey with conspicuous colouration. However, this is not likely because being conspicuous without any defence offers no protection and simply makes the prey more visible to predators. On the other hand, without following exclusive pathways, unprofitability and conspicuous signals could evolve together. Much of the literature on adaptive colouration has been particularly concerned with the predator–prey relationship. It has long been recognized that defended prey tend to be conspicuous and that aposematism arises because predators more readily learn to avoid attacking defended phenotypes when they are conspicuous. In order to fully understand aposematism there should be a clear picture of the evolutionary history behind the present behaviours.[189] Many studies on aposematism have been descriptive, or conducted strictly from the ecological or behavioural points of view. Aposematic colouration is common in most opisthobranch taxa and is more predominant in tropical faunas than in temperate ones, though at least one study disputed the role of pigments in aposematic colouration of tropical sponges.[190,191,192] Resolving the debate on the evolution of aposematism will require experiments and observation of predation on aposomatic species in the wild.

Conspicuous signals are most often bright colours usually presented in banded or contrasting patterns. Bright colours may advertise to potential predators that a species is distasteful, toxic, unpalatable, vicious or difficult to capture. For a colour pattern to be aposematic, predators must be able to see the colour or at least detect contrasting patterns of shading. Conspicuous warning signals of unprofitable prey are a defence against visual hunting predators. Warning colours are usually, but not always, conspicuous and consist of bright colours of red, yellow or white, often in combination with black, and are not intended to camouflage, but to make the animal more conspicuous. They work because predators learn to associate unprofitability with bright colouration and because strong signals are detectable and memorable.[193] However, many species that can be considered defended are not very conspicuous; they have weak warning signals.[194]

The co-occurrence of conspicuous colouration and noxious characteristics suggests that aposematism is a common predator deterrent in many coastal marine species. The organisms use warning displays for defence and better survival, a strategy commonly found among nudibranchs (Opisthobranchia, Mollusca), particularly in many species of the nudibranch families Chromodorididae and Phyllidiidae (colourful dorids or sea slugs). Colour has played a major role in the adaptive radiation and evolution of opisthobranch gastropods, which are among the most brilliantly coloured organisms inhabiting the world's oceans. They have spectacular and obvious colour patterns for warning potential predators, are known to have reduced or entirely lost their shells independently within several distinct lineages, and evolved (or acquired from dietary sources) toxins that provide protection from predation.[105,195] Even though nudibranchs possess no protective shell and seem to be easy prey for fish or other predators such as crabs, reports of predation (except by other nudibranchs) are virtually non-existent.[196] They can also be coloured in such a way as to warn predators that conspicuous colouration is often associated with the presence of toxic or defence substances. Studies on shell-less molluscs led to the theory that co-evolution with toxic foods had allowed them to lose their shells over evolutionary time.[74,126] Many chromodorid nudibranchs take distasteful chemicals from the sponges they feed on and store them in special glands (repugnatorial glands), which are located around the mantle edge. Contact with these antifeedant compounds is an extremely unpleasant dining experience and visual predators will quickly learn to avoid them by recognition of their distinct and bright colour patterns. Many aposematic species have evolved to live in close proximity to others of their species; one possible advantage of this is that it makes the aposematic signal more effective by generating a greater aversion in predators. Sponges can be toxic to fish, and distasteful due to tough fibres, spicules and toxic substances.[197] Not only can organisms change their colour to elude the enemy, they can also emit dye to detour them.[22,198] Even though no explanation other than aposematism has been proposed for the colouration of marine gastropods that is consistent with what is known of their biology, it should be noted that no experiments on these organisms have conclusively demonstrated aposematism.[199–201] The different categories of colour in opisthobranch molluscs have been reviewed.[20,21,202]

The possibility that bioluminescence functions as an aposematic signal has been suggested.[199] Bioluminescence in the Basket brittle star, *Ophiopsila riisei*, functions as a facultative aposematic signal (to deter crab predators) and is common among generalised grazers, while more specialised predators often exhibit special resemblance to their prey.

Toxicity or reduced palatability is due to a variety of organic compounds, such as the isonitriles kalihinol A and B **69a+b**, furans (*e.g.* furospongin **70**), and inorganic compounds (*e.g.* sulfuric acid); most of these compounds are derivatives of chemicals produced by the prey. Only a few species manufacture toxins *de novo*.[203–205] Laboratory feeding assays with fish and food pellets containing organic extracts of several species of demosponges from the reef, mangrove, and grass bed habitats indicated that most sponge species yielded deterrent extracts but there was considerable inter- and intra-specific variability in deterrency suggesting that, at least for some species, some individuals may be chemically undefended. Reef sponges generally yielded more deterrent extracts than sponges from other habitats, and

69a   **69b**

70
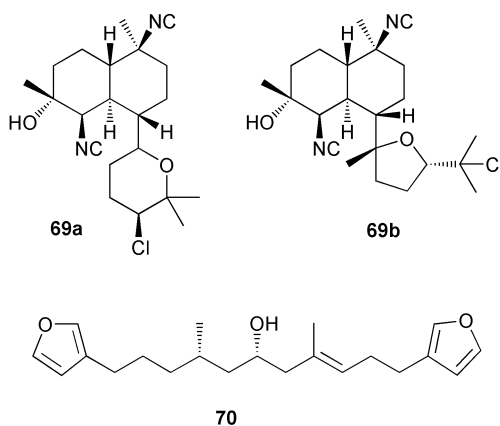
there was no relationship between sponge colour and deterrency, suggesting that sponges are not aposematic.[205,206] Feeding fish with brightly coloured larvae, Young and Bingham[207] addressed both issues of chemical defence and aposematism. They and other investigators[173,208] have demonstrated how widespread aposematism might be among marine invertebrates, that rejection of larvae by fish was chemically based, and that coloured (conspicuous) larvae are more likely to possess chemical defences.

11.2 Advertisement

Many species, including marine organisms, use colour-based visual signals to attract mates, warn rivals, or confuse predators. Often, bold designs accompany an unpleasant taste or dangerous venom. Aposematic colouration works by advertising unpleasant qualities to potential predators. However, aposematism is not the only reason for bright colouration of marine organisms – bright colours also have other functions. For example, advertisement by bright colours or conspicuous patterns serves various purposes in coral reef organisms. Various life stages of organisms such as fish involve colour changes. Animals also change colour to signal different moods. Some fish are pale when frightened and darken in anger or in sexual excitement. The octopus can turn black when in danger. Colouration also functions by differentiating between sexes and aiding in selection of potential mates. Males are mostly more brightly coloured than females – in some species, the colours and patterns are present only during the breeding season in order to attract the female. Other courting rituals such as intricate dances accompany these colour changes.[199,200,202] The ability to change skin colour patterns allows squid to court mates, challenge rivals, and communicate with one another. A male Caribbean reef squid, for example, splits into two patterns during courtship; its coppery right side is oriented toward the female, and its silvery left side is to ward off rival males.[209] Because physical and chemical colouration is determined genetically, traits are passed on from parent to offspring.

Many marine animal species are genetically polymorphic in colour. Visual selective predation is commonly suggested as a mechanism for maintaining colour polymorphism in prey species and may, for example, result in correlations between colour frequencies and the background colour. Such correlations are commonly observed in some polymorphic gastropods.[210]

11.3 Crypsis

Many organisms in the ocean have the ability to hide or conceal themselves, a phenomenon referred to as crypsis or homochromy. Some organisms use methods of concealment to avoid being discovered by potential predators and prey. There are three main forms of crypsis: disruptive colouration, camouflage, and colouration which mimics that of the host.

Disruptive colouration occurs when organisms are spotted, striped or have other types of patterns to make their outline difficult to distinguish. These patterns are useful to the marine organism because they often blend in well with the surroundings and aim to obscure the true form of the animal by partly blending with the background and distracting the attention of the viewer.[211] Crypsis through background matching requires that the sizes and shapes of the pattern elements should closely resemble those of

the visual background.[212] Animals like the octopus, cuttlefish and the squid can change their colour depending on the situation that they are in. It is most often facilitated by the resemblance of the organism to the background.

Another common method of crypsis or concealment is countershading (pelagic colouration). Countershading, the gradation of colour from dark on the dorsal (upper) side to light on the ventral (lower) side is generally considered to have the effect of making organisms difficult to detect. The mechanism that facilitates this form of crypsis is often considered to be concealment of shadows cast on the body of the animal, but there is no conclusive empirical evidence for this function. However, highly refined adaptations in some marine organisms to match the different background light conditions against which they are set when viewed from different aspects strongly suggest an adaptive advantage to countershading in these environments.[213]

Although most transparent species reside in the pelagic zone of the ocean, some benthic, neustonic (minute, surface-dwelling) organisms have transparent members. Among the transparent animals are shrimp, octopuses, polychaete worms, the hydrozoan Portuguese Man-of-War, and copepods.[214] Transparency is one of the few forms of camouflage, but it is a very successful method in environments that offer no surfaces to match or hide behind, and appears to have evolved for passive crypsis.[215] In the pelagic environment, the most prevalent form is complete transparency, achieved by reflection of most (or all) of the visible wavelength of light, and counter-illumination by bioluminescence.[216]

In dermasal colouration, some species have similar colours and designs that blend in with the bottom of the substrate. Yet some others have the capacity to bear a general resemblance to vegetation, which is termed vegetal colouration. Organisms also show colour resemblances to substrates that consists of stones, shells, and small algae, individually or in combination. Marine invertebrates employ blue and yellow carotenoproteins for camouflage or in mating rituals. Sometimes a symbiont can help the host to camouflage. The luminescent symbiont *Vibrio fischeri* is housed in a complex light organ within the mantle cavity of the Hawaiian bobtail squid *Euprymna scolopes*, and the bioluminescence by the symbiont helps the squid to camouflage itself.[217]

For many reasons and in many ways, some marine organisms change their colour and sometimes their shapes to disguise themselves. Many animals, such as the decorator crab, Pederson's cleaning shrimp and the sea horse change their whole colour and appearance to hide from predators. The nudibranch *Discodoris indecora* shows a perfect camouflage on its prey, the dictyoceratid sponge *Ircinia variabilis*. The shape and colour of the nudibranch are remarkably similar to that of the sponge, which is widespread in shallow waters of the Mediterranean Sea. While feeding on sponges, the nudibranchs sequester the effective chemical armoury of their prey, which is subsequently employed for their own protection.[139,196]

The cuttlefish *Sepia officinalis* can produce a variety of body patterns for camouflage on natural substrates, and they can blend into the surroundings almost as fast as they can move.[218,219] On mixed light and dark gravel, they show disruptive body patterns for camouflage.[220,221] The recently discovered rare mimic octopus impersonates the shape, posture and movements of various other animals. The octopus' banded colouration copies the dramatic banding of many dangerous or poisonous sea animals such as

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sea snakes, lionfish and flatfish.[222] There has been considerable debate regarding the function of coloured compounds in the shells of marine organisms. The red colouration of *Calloria* spp. (Brachiopoda) is due to a carotenoprotein that occurs within the crystals of the shell. Equivalent pigments from mollusc shells are considered to be a by-product of metabolism.[223–225] Red colouration permeates the body tissues of living brachiopods, as well as the shell.[226] Such colouration in brachiopods as well as in other taxa is thought to have a photo-protective function. Red pigmentation of animal shells may have a protective role by warning potential predators that the brachiopod tissues may contain toxin, or by functioning as camouflage.[50,227] Many species of arboreal marine snails are polymorphic with regard to shell colour, with morphs that correspond in both colour and frequency to those predominant in the habitat. A combined effect of selection for crypsis and apostasy has been suggested as the most likely reason.[228] Sea hares (Family Aplysidae, Order Anaspidea) are herbivorous opisthibranch molluscs that acquire and store algal metabolites that may act as chemical defences against predators. Like many other opisthobranchs, sea hares have multiple defenses against predation including physical, behavioural and chemical strategies. Physical and behavioural strategies include crypsis and movement, and crypsis is the most common and primary means for avoiding predators. One of the most common features of most sea hares is that the organism produces clouds of reddish purple "ink" when disturbed, whose function is yet to be determined.[88,202,203]

11.4 Mimicry

In some geographical areas, groups of unrelated nudibranchs such as the red-spotted chromodorid species found in south-eastern Australia, and polyclad flatworm species found in California, have evolved very similar colour patterns, so that they share the task of teaching the predator to leave the colour pattern alone. A number of harmless snakes closely mimic the bright warning colouration of the coral snake – the most poisonous snake in the United States. The colour pattern of a few nasty distasteful species is copied by other quite edible species. These species gain considerable protection from predators that have learnt from experience to leave the distasteful species alone. This phenomenon where one organism (the mimic) evolves to resemble another species (the model) such that these two organisms are confused by a third organism (the receiver) for gaining some biological benefit is called mimicry. The concept of disguise involves not only colour, but also body shape and behaviour. Warning colour and mimicry form part of the general topic of defensive colouration. Mimicry in the ocean is especially deceptive because of the capabilities of organisms, such as molluscs, to grow appendages and go through quick changes. An evolving mimic takes advantage of previously evolved communication signals and responses between organisms. To be successful and beneficial to the mimic, the model should be an abundant species whose noxious characteristics have left a lasting impression on predators.

There are four major forms of mimicry utilised by both predator and prey: Batesian mimicry, Mullerian mimicry, aggressive mimicry and self-mimicry. Mimicry refers to the similarities between animal species; camouflage refers to an animal species resembling an inanimate object.

In *Batesian mimicry*, first described in 1852 by the British naturalist Henry Bates, the mimicking organism has evolved some features of a poisonous organism but is not poisonous itself – a Batesian mimic cheats.[229] In the case of two unrelated, but similarly marked families of Brazilian forest butterflies, one of which (the model) was poisonous to birds and the other (the mimic) palatable, the latter survived because of their resemblance to the poisonous family. Mimics usually copy the aposematic colouration of the model species. In this kind of mimicry, the mimicking organism has evolved some features of a poisonous organism but is not poisonous itself. This is essentially equivalent to camouflage.

In 1878, the German zoologist Fritz Muller proposed an explanation to Bates' paradox, in describing *Mullerian mimicry*. In Batesian mimicry, one species is distasteful and another one is palatable, while in Mullerian mimicry both species are distasteful. While Bates had observed a resemblance among several unrelated butterflies all of which were inedible, Muller realised that the explanation might lie in the advantage to one edible species in having a predator learn from another. Once the predator has learned to avoid the particular conspicuous warning colouration with which it had its initial contact, it would then avoid all other similarly patterned species, edible or inedible. Mullerian mimics gain maximum protection when all individuals have the same signal (signal standardisation). This tendency of inedible and noxious species to evolve to have the same or similar warning signals such as the same pattern of bright colours is called Mullerian mimicry.[230] This form of mimicry refers to two unpalatable species that are mimics of each other with conspicuous warning or aposomatic colouration. Thus all the mimics share the benefits of the colouration, since the predator, after a few bad experiences, will recognise the colouration of an unpalatable group.

In *aggressive mimicry*, the organism mimics a signal that is attractive or deceptive to its prey.[202,229,231–234] Aggressive mimicry usually is seen in skin biters imitating cleaner fishes. Cleaners are not often preyed upon and other fishes that mimic their colour patterns and behaviour can gain protection. The red and black colouration of deep-sea marine organisms appears black at depth, and black blends in, rendering these organisms invisible to predators.

In some cases, as in *self-mimicry*, colouration mimics a particular region or microhabitat of the host. Self-mimicry is a misleading term for animals that have one body part that mimics another to increase survival during an attack or helps predators appear innocuous. Host mimicry may be further enhanced by morphological or behavioural adaptations. In all these instances, a mimetic relationship is postulated.[235] Reimchen reported the first example of gastropod–polychaete mimicry and presented evidence for the overwhelming role of visual predators and natural selection in the evolution of shell colour among intertidal prosobranch Littorinamariae.[235] There are only a few examples of aggressive and self mimicry in the marine environment.[229]

12 The defensive role of pigments

The high frequency of bioactive components in the primitive filter-feeding sponges is interpreted as chemical defence against environmental stress factors such as predation, infection, overgrowth by fouling organisms or competition for space.[236] These toxic or deterrent sponge metabolites are found in habitats such

as coral reefs that are characterised by intense competition and feeding pressure. Further support for the adaptive significance of sponge constituents is derived from the observation that sponges which grow in exposed places are usually more toxic than those in unexposed places.[237] Early studies concluded that the incidence of defensive chemicals was much higher at tropical than temperate latitudes. Tropical–temperate comparisons led to a "latitudinal" hypothesis, suggesting an inverse correlation between the incidence of chemical defence in marine invertebrates and latitude. Whereas sponges or the symbionts associated with them are believed to synthesise these metabolites, gastropods and nudibranchs sequester this "chemical armoury" from their prey, which is subsequently employed for their own protection. Some nudibranchs, however, have become independent of this interspecific flow of natural products and are able to accumulate defensive compounds through *de novo* synthesis.[238]

Porifera (sponges) are the dominant phylum on the Antarctic benthos, and members of this phylum have been shown to elaborate sea star feeding deterrents, inhibitors of fouling or infectious organisms, and metabolites which mediate predation by moult inhibition. Antarctic sponges have some unique attributes. They are remarkably diverse – some are related to deep-sea sponge species, but some others to those from South America. Among the common members of the sponge community are several conspicuously coloured species. *Isodictya erinacea* (polychaete sponge) and *Dendrilla membranosa* are bright yellow sponges lacking structural defences such as spicules, and the deep green demosponge *Latrunculia apicalis* is not preyed upon by the spongivorous sea star *Perknaster fuscus*. This ecologically relevant sympatric sea star is a major predator of the sponges in this environment.[239–243] For example, the fast-growing, potentially space-dominating sponge *Mycale acerata* is kept in check by this sea star. The soft-bodied, fire-red sponge *Kirkpatrickia variolosa*, a minor component of the diet of the sea star *P. fuscus*, is the most striking sponge in the Antarctic benthos. *Suberites* sp., with a yellow appearance, is a common Antarctic sponge. *Asbestopluma hypogea* (Carnivorous sponge), *Cinachyra subterranean* (Globular sponge), *Cinachyra antarctica* (Spiky sponge), *Clathria nidificata* (Orange dendritic sponge), *Dendrilla antarctica* (Cactus sponge), *Iophon radiatus* (Epibiotic sponge), *Hexactinellida* sp. (glass sponges), and *Leucetta leptoraphis* (Rubber sponge), are other examples of brilliantly coloured Antarctic sponges. The colours of the Antarctic sponges can be white – light tan, bright yellow, greenish-yellow, green, dark brown, violet, or (in a few cases) pink. The pigments which bestow this bright colouration are themselves various shades of orange, yellow and purple. Antarctic benthic marine invertebrates have no apparent reason to be brightly coloured, yet they are, suggesting that pigmentation could not be driven by aposematism.[200,205,239,240,243] The presence of this bright colouration is intriguing in a marine system where grazing pressure by visually oriented predators such as fish is generally lacking. In contrast to predictions that global patterns of predation should select for an inverse correlation between latitude and chemical defences in sponges, such defences are not uncommon among Antarctic sponges.[26,241–243] The characterisation of chemical defences and the presence of bioactive pigments from most highly pigmented benthic Antarctic invertebrates raise the intriguing questions of the role of colouration in such an ecosystem, and whether evolutionary selection for, or retention of, pigmentation

has been driven by predation pressure.[239,243] Colouration in these sponges may be the result of relict pigments originally selected for aposematism or UV screening, yet conserved because of their defensive properties. Pigments in other marine organisms and terrestrial plants are almost exclusively employed in energy capture or to serve as antioxidants, but these are unlikely roles for pigments of benthic marine invertebrates. Conversely, there is little evidence on whether pigments from tropical or temperate benthic marine invertebrates may serve as antifeedants. The distribution of known metabolites in these organisms differs considerably from that of the general marine realm.[239–243] Most of the secondary metabolites described from Antarctic organisms are not found in congeners from temperate and tropical regions. Most of them are vulnerable to predation, especially by sea stars. Recent laboratory studies have indicated that these pigments in Antarctic sponges are themselves bioactive feeding deterrents, and defend the organisms from predators or fouling organisms.[239,243] The hypothesis is supported by the bioactive properties of pigments present in them. Among the pigments characterised so far are the variolins, *e.g.* variolin B **48** from *Kirkpatrickia variolosa*, bearing a very unusual pyridopyrrolopyrimidine ring system. This is a group of alkaloids represented by the discorhabdins, *e.g.* discorhabdins A and C **71a+b** (present in *Latruncula apicalis*), suberitenones A and B **72a+b** (the major metabolites of *Suberites* sp.), and the yellow isoquinoline pigment **73** from *Dendrilla membranosa*. The discorhabdins account for the green colouration of *L. apicalis*,[244] and discorhabdin C **71b** is a potent mammalian cytotoxin, emphasising its bioactive nature.

**71a**   **71b**

72a   **72b**
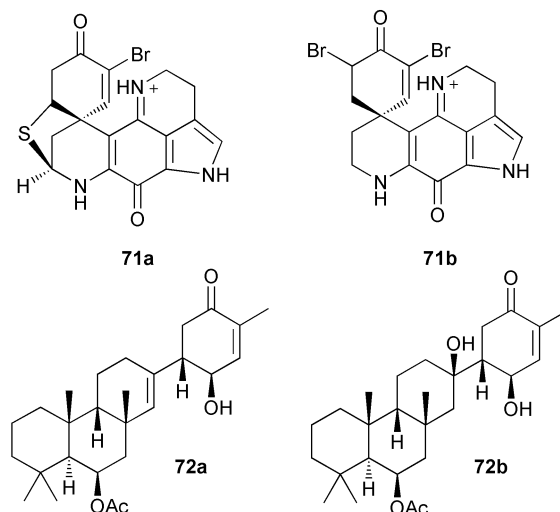
The pigments variolins, discorhabdins, the isoquinoline metabolite from *Dendrilla membranosa*, and to a lesser extent, the suberitenones, display ecologically relevant bioactivity, including tube-foot retraction (indicative of feeding deterrence) and antimicrobial activity. These pigments displayed bioactivity towards their common enemy, the sea star *Perknaster fuscus*, which lacks visual orientation.[243,245] They serve as defensive agents, both toward predators and potential infectious agents. Discorhabdin C is perhaps the strongest sea star deterrent studied to date.[243] Sea stars, the specialist predators of the Antarctic sponges, often feed by extruding their cardiac stomachs over their prey. Because of this unique stomach extension, feeding mechanisms and feeding assays typically used with other predators are less appropriate for sea stars. The tube-feet are chemosensory, used by the sea

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star for, among other things, assessment of prey suitability – contact of the tube-feet with sponge extracts elicits a characteristic chemosensory response.[243] It is generally accepted that tube-feet are a primary site for chemical reception in echinoderms and that tube-foot chemoreception in sea stars is primarily a defensive response.[245] The quinoline pigment **73** present in *D. membranosa* is inhibitory towards a water-column bacterium.[246] It is among the few Antarctic sponges known to produce terpenes.[243] Cytotoxic erinacean **74** has been isolated from *Isodictya erinacea*, and ecologically relevant concentrations of *p*-hydroxybenzaldehyde from this bright yellow sponge are feeding inhibitors.[239,243] The yellow pigment eribusinone **75**, a tryptophan catabolite, has been isolated from the sponge *I. erinacea*. Tryptophan catabolites are involved in crustacean moult regulations. Moult regulation as a potential mechanism of chemical defence in a marine realm has been demonstrated. Eribusinone **75** from *I. erinacea* was studied as a moult inhibitor due to its structural similarity to the crustacean moult inhibitor 3-hydroxykynurenine **76**. Preliminary feeding experiments conducted with a carnivorous predator, the amphipod *Orchomene plebs*, on a diet enriched with eribusinone, led to significant decrease in moulting events and concomitant increase in mortality.[243]
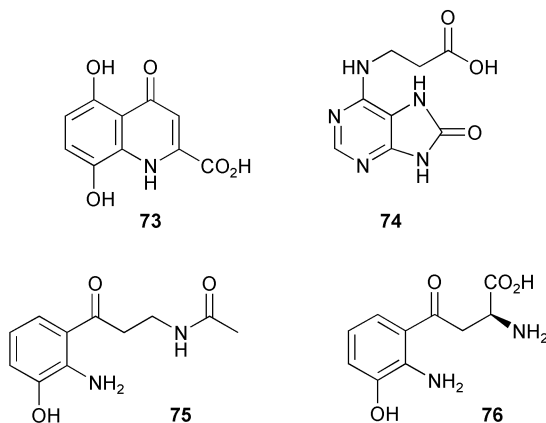

The ovaries, eggs, embryos and larvae of some of these Antarctic benthic invertebrates are unpalatable or chemically defended from sea star, sea anemone and amphipod predators.[241,242] In one interesting twist, it has been observed that a sponge pigment mimicked a chemical involved in regulating moulting of an amphipod known to prey on the sponge. When the amphipod eats the sponge the chemical pigment prevents moulting and the amphipod eventually dies.[239,241–243] Studies on Antarctic sponges has thrown new insights into the functional role of pigments and the ability of sponges to sequester metabolites, and a new mechanism of chemical defence has been described.[239,243]

Chemicals released when organisms are attacked can serve to mark a location as dangerous to conspecifics. Many gastropods leave a slime trail behind them as they move, which can allow for easier location by conspecifics in search of mates. However, when sufficiently molested (as by a predator), the nudibranch *Navanax inermis* secretes a mixture of bright yellow chemicals, the navenones, (*e.g.* navenones A and B **77a+b**) into its slimy trail, displays alarm pheromone properties and causes an avoidance response in trail-following conspecifics.[247,248] The lignarenones, *e.g.* lignarenones A and B **78a+b**, isolated as pale yellow gums from the mantle of the mollusc *Scaphander lignarius* and structurally related to the navenones, did not show similar properties.[249]
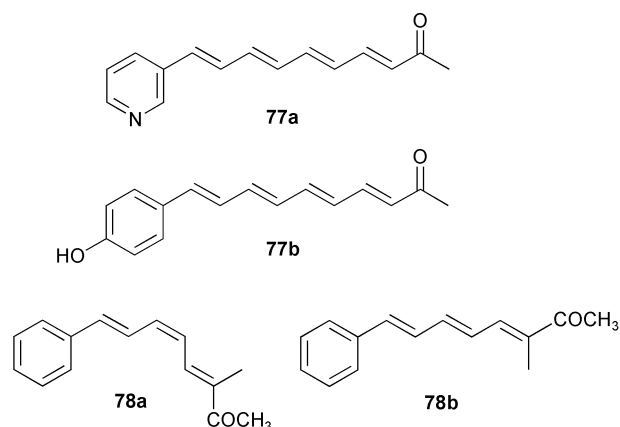
Tambja abdere, the greenish-coloured nudibranch with mottled patches of yellow and bluish-ocre splattered through the body, sequester tambjamines A, B, C, E and F **79a–e** (belonging to the 4-methoxypyrrole family of natural products), from its bryozoan prey for the same purpose, and to deter fish. *Holothuria atra*, when handled, releases a burgundy-coloured toxin copiously into the surrounding water, which is lethal enough to kill polychaetes and flatworms and immobilise cone snails (*Conus*).[22,198] The red-coloured sponge *Latrunculia magnifica* found in the Red Sea, when squeezed, exuded a reddish fluid that caused fish to flee immediately from the vicinity, and when the juice was squeezed into aquaria, fish (*Gambusia affinis*) died within a few minutes. Chemical analysis afforded the macrocyclic light yellow alkaloids, the latrunculins (*e.g.* latrunculins A and B **80a+b**), which were responsible for the strong ichthyotoxicity of the sponge.[196] The unusual sesterterpenoids palinurin **57** and variabilin **81** are the
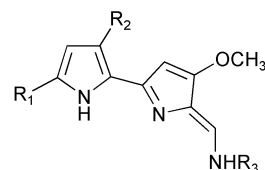
79a R₁ = R₂ = R₃ = H
79b R₁ = Br = R₂ = R₃ = H
79c R₁ = H, R₂ = H, R₃ = CH₂CH(CH₃)₂
79d R₁ = H, R₂ = H, R₃ = CH₂CH₃
**79e** R₁ = H, R₂ = H, R₃ = CH₂CH₂Ph
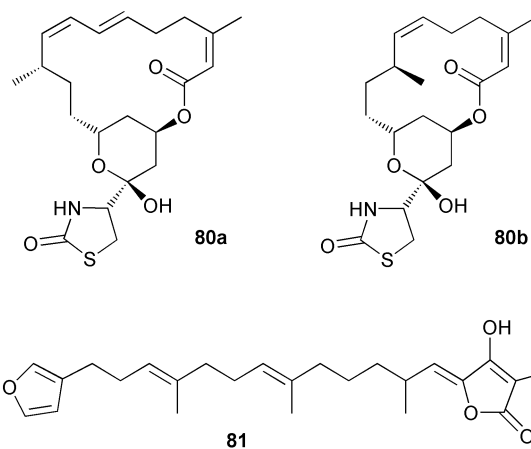
dominant metabolites in *Ircinia variabilis*. When disturbed, the sponge secretes copious amounts of a substance that contains large amounts of both metabolites. The defensive role of palinurin and variabilin is strongly supported by their anatomical location.[139]

13 Physiological activities of pigments

Several studies of the chemical defences of marine invertebrates point to an increased interest in understanding the ecological rationale for the production of these compounds, and their biological activities. Pigments in marine invertebrates and their distribution and functions seem to differ between invertebrate groups. It is possible that some of the bright colours observed today are relics from the past, when the colours served an adaptive function that now has been lost due to changes in environmental conditions, or extinction of predators that once were deterred by the colour patterns. For example, alkaloids (especially the indole and bis(indole) alkaloids comprising the yellow pigments from sponges, ascidians and hard corals) play an important role as the defence of the host animal against predation, and have shown a broad spectrum of biological activity. It is proposed that the presence of these pigments is linked to the biosynthetic activity of the symbiotic bacteria.[74–76] Marine organisms, the nudibranchs in particular, which are shell-less (and hence seemingly defenceless), and conspicuously coloured, have invariably been found to contain compounds identical or similar to those found in their diet.[250] These compounds are accumulated on a functional basis, and it is possible that nudibranchs fractionate the best chemical defence compounds from their diet. This biological rationale suggests that potent defence compounds have other uses. Indeed, the unusual blue tetrapyrrole pigment **13** from the nudibranch *Nembrotha kubaryana* was found to be a potent antimicrobial agent. In addition, tambjamines A, B, C, E, and F **79a–e** isolated from this organism showed antimicrobial, antitumour and immunosuppressive activities.[88] Several guaiazulenes and related sesquiterpenoids, well recognised for their blue and purple colours, have been reported to exhibit various bioactivities, including antibacterial, antimicrobial, cytotoxic, antitumour, and immuno-regulating activities, as well as inhibitory activity against cell division of fertilised sea urchin and ascidian eggs.[115,251–253] Two new carotenoids isolated from the sponge *Phakellia stelliderma* showed mild cytotoxicity against mouse leukemia cells.[83] The red pigment fascaplysin **59** (a metabolite of *Fascaplysinopsis* sp.[254]), arcyriaflavin **82** and the related staurosporine aglycone **83** (from *Eudistoma* sp.,[255] a tunicate), the pale yellow disulfide polycarpine **84** (isolated from the tunicates *Polycarpa clavata*[256] and *P. aurata*[257]), the discorhabdins **71a**+**b** and related pigments from the sponge *Latrunculia apicalis*,[258] the meridianin alkaloids (*e.g.* **58** from the tunicate *Aplydium meridianum*[133]), coscinamide alkaloids (*e.g.* **85** from the sponge *Coscinoderma* sp.[133]) and topsentin alkaloids (*e.g.* **50** from the sponge *Spongosorites genitrix*[76]) have been shown to be cytotoxic. The meridianins are a new family of protein kinase inhibitors.[133]

A marine gram-negative aerobic bacteria that produced cytotoxic lemon-yellow chromopeptide pigments that inhibited the development of sea urchin eggs has also been isolated from the Australian sponge *Fascaplysinopsis reticulata*.[259] The discorhabdins, *e.g.* **71a**+**b**, are often cytotoxic, bear antitumour and antiviral properties, and have broad-spectrum antibiotic activity
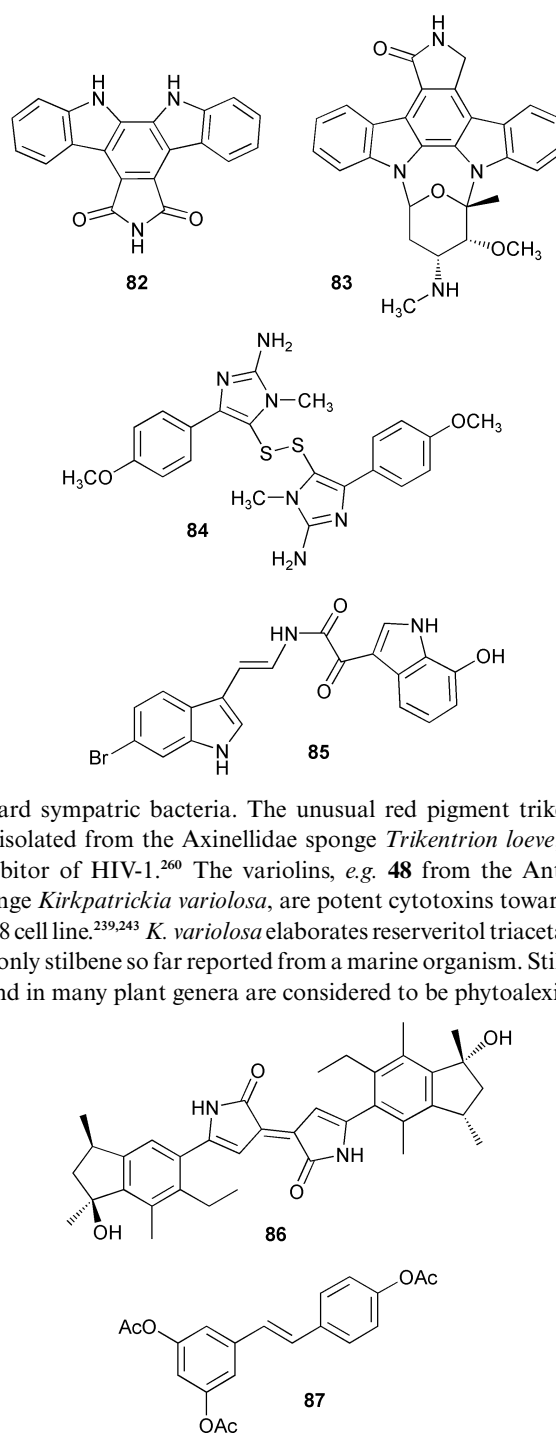
toward sympatric bacteria. The unusual red pigment trikendiol **86**, isolated from the Axinellidae sponge *Trikentrion loeve*, is an inhibitor of HIV-1.[260] The variolins, *e.g.* **48** from the Antarctic sponge *Kirkpatrickia variolosa*, are potent cytotoxins towards the P388 cell line.[239,243] *K. variolosa* elaborates reserveritol triacetate **87**, the only stilbene so far reported from a marine organism. Stilbenes found in many plant genera are considered to be phytoalexins.

Among the ecologically relevant natural products, the yellow pigment eribusinone **75** from the Antarctic sponge *Isodicta erinacea* appears to be a tryptophan catabolite, and behaves much as other such catabolites in regulating crustacean moulting.[239,243] An unusual alkylated purine, with no currently understood functional role, has also been isolated from the organism. Antibiotic bromophenols are present predominantly in the distal regions of the polychaete worm *Thelepus extensus*, suggesting a role in fouling and/or infection control. Arcyriaflavin **82** and the related staurosporine aglycone **83** (from the grey-coloured tunicate *Eudistoma* sp.), aaptamine **88** and isoaaptamine **89** (both

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pale brown in appearance, from the sponge *Aaptos aaptos*) are protein kinase C inhibitors, as are the oroidin alkaloids *Z*-axinohydantoin **52** and debromo-*Z*-axinohydantoin **90**, obtained as minor metabolites from the sponge *Stylotella aurantium (agminata)*.[169] This organism fluoresces and its colour varies from yellow to orange. Similarly, the spongiacidins B and D **53a+b** (another group of metabolites from the oroidin group) from a *Hymeniacidon* sp. sponge,[130,170,203] and the rhopaladins **91a+b**, bis-alkaloids from a blue translucent sea squirt, *Rhopalaea* sp., are c-erbB-2 kinase and cyclin-dependent kinase 4 inhibitors.[261]
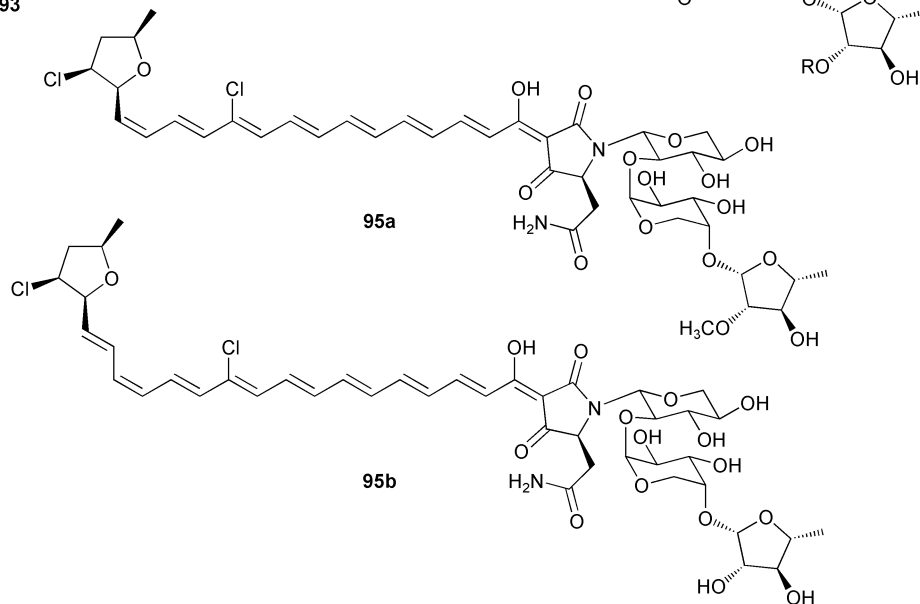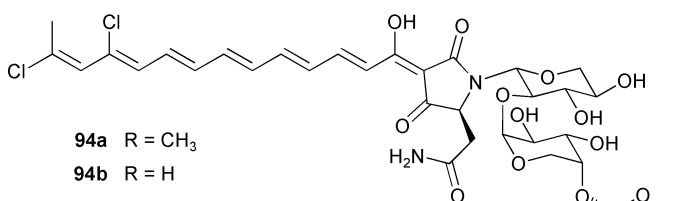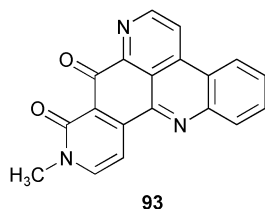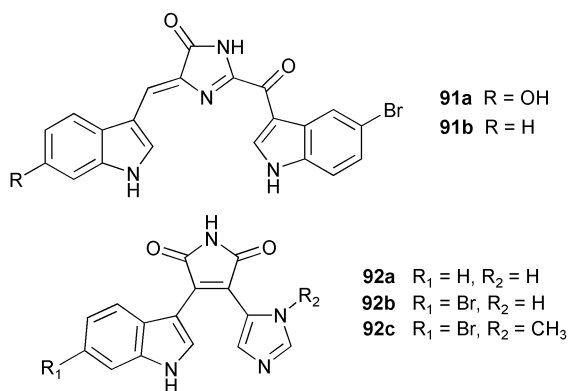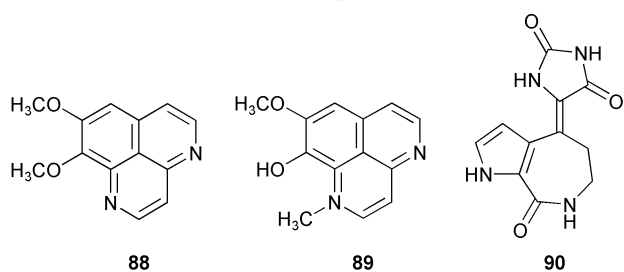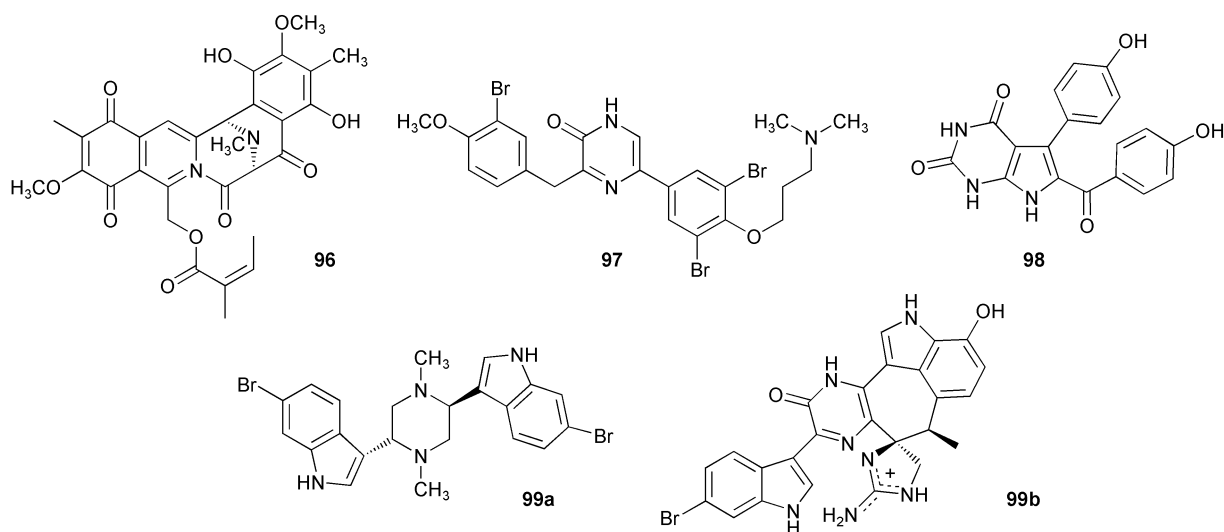
The ascidian *Didemnum conchyliatum*, which was collected from sea grass blades in mangrove habitats, contained the indole alkaloids didemnimides **92a–c**, which inhibit predation by fish, while *Didemnum granulatum* contained G2 cell cycle check-point inhibitors.[261–263]

The insecticidal activity and cytotoxicity of kuanoniamines **37** from a Micronesian sponge, *Oceanapia* sp., has been reported.[264] The ascidian *Pseudodistoma aureum* contained the β-carboline eudistomin **62b**, a calmodulin antagonist also isolated from the tunicate *Eudistoma glaucas*.[265] The pyridoacridine neoamphimedin **93**, a constituent of the sponge *Xestospongia* sp., is a topomerase II inhibitor that catenates DNA.[266] The tetramic acid glycosides, aurantosides A and B, **94a+b**, isolated from the lithistid sponge *Siliquariaspongia japonica* possess antifungal activities,[267] while the related tetramic acid glycosides rubrosides A and B **95a+b**, present in the same organism, induce large intracellular vacuoles in 3Y1 rat fibroblasts.[267]

The Caribbean tube sponge *Cribrochalina* sp. contained the isoquinoline alkaloid cribrostatin **96**, a minor antimicrobial agent, and a *Suberea* sp., a sponge, contained a cytotoxic pyrazin-2-(1*H*)-one, ma'edamine **97**. A phosphodiesterase inhibitor from *Eudistoma rigida* has been named rigidin **98**.[268,269] Dragmacidin B **99a** and D **99b** is an antiviral bromoindole alkaloid from a Mediterranean sponge, *Halicortex* sp.

Keramidine **100**, the antagonist of the serotonergic receptor of the rabbit aorta, is present in a species of *Agelas*,[270] a sponge. The quinoline pigment **73** in *Dendrilla membranosa* (a pale brown – deep red sponge inhabiting the Antarctic and South George Island) along with the alkaloids isolated, are likely to be responsible for much of the anti-predator, antibiotic and antibacterial activities reported for the sponge.[271,272] The bis(imidazole) alkaloid corallistine **101** from the deep-water, lithsid sponge *Corallistes*

88   89   90

91a R = OH
91b R = H

92a R₁ = H, R₂ = H
92b R₁ = Br, R₂ = H
92c R₁ = Br, R₂ = CH₃

93

94a R = CH₃
94b R = H

95a

95b

96   **97**   **98**

99a   **99b**

fulvodesmus, and the naamidines and isonaamidines **102a–f**, 2-aminoimidazole alkaloids isolated as yellow gums from the bright yellow calcareous sponge *Leucetta chagosensisis*, possess anthelmintic and antiparasitic properties.[126,273]

100   **101**
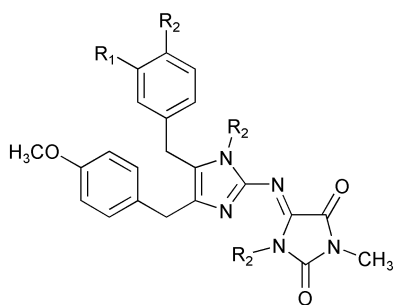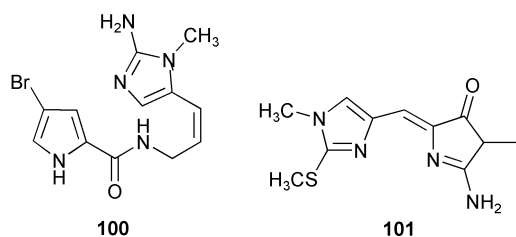
102 a-d R_1 = H or OH, R_2 = H or CH_3
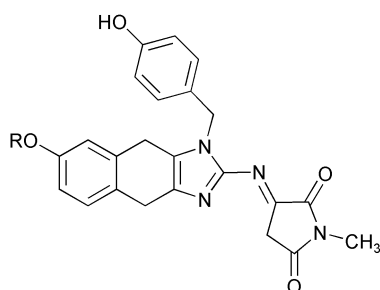

102 e-f   R = H or CH_3

The immunosuppressive activity and weak cytotoxicity of the echinclathrines A–C **103a–c**, a new class of yellow pyridine

alkaloids, have been demonstrated.[274] The intensely coloured extract from the bright orange sponge *Phakellia stelliderma* afforded two red pigments, 19′-hexanoyloxymytiloxanthin **104a** and 19′-butanoyloxymytilioxanthin **104b**. In an *in vitro* assay with P388, **104a** showed an IC_{50} of 10 µg mL^{-1}.[274] The antineoplastic indole alkaloid aplysinopsin **105** was isolated simultaneously from six species of sponges: five were *Thorecta* species and the other *Verongia spengelii*.[118]

Scytonemin **66**, topsentins (*e.g.* **50**), and debromohymenialdisine **90** have provided several lead compounds in the field of inflammation, rheumatoid arthritis and osteoarthritis respectively. There is evidence to show that the topsentins are potent mediators of immunogenic inflammation, and that both topsentin and scytonemin are most potent inhibitors of neurogenic inflammation. Debromohymenialdisine **68** has been patented as a protein kinase C inhibitor.[74,126]

14   Pigments and metal ion chelation

Nature can be considered to be an imaginative coordination chemist. It synthesises small molecular weight chelates with most interesting combinations of donor groups, as well as large biopolymers with intricate structures that contort to produce an array of donors, tailored to encapsulate specific metals. Some of the marine natural products (Table 1) appear to have structural features such as polar functional groups in potentially chelating arrangements, macrocyclic "cavities" and geometries apparently tailor-made for wrapping around metal ions that make them ideal for chelating with metals.[275,276] The siderophores are some of the best-known families of strong small chelates. A broad range of molecules, including macrolides such as the bistratamides, lissoclinamides, patellamides, ulapualides, ulicyclamide, bastidines, and many pigments, with a variety of donor groups capable of metal binding, have been extracted from a range of marine organisms including the sponges, ascidians and, to a lesser extent, from nudibranchs.[275,276] The donor groups vary from low molecular weight bidentate and multidentate chelates to proteins containing the catechol side groups. The pyrrole group and related nitrogen heterocycles are frequently found in many pigments, and sometimes in association with a peptide group,

103a

103b R = Ac
103c R = H

104a R = (CH₂)₄CH₃
104b R = (CH₂)₂CH₃

105
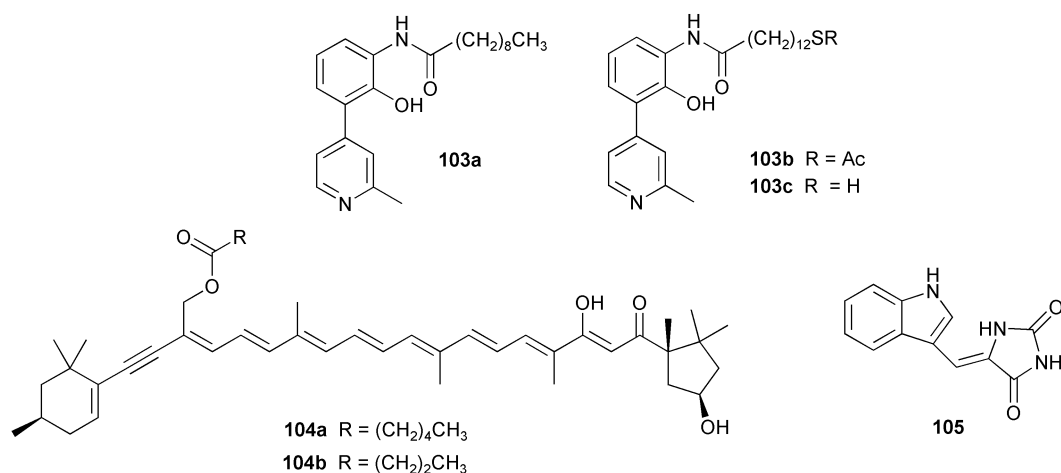
for example, the deeply red-coloured pigments prodigiosin **106**, metacycloprodigiosin **107** (containing a 4-methoxy-2,2′-bipyrrole moiety), oroidin **108**, tambjamines **79a–e**, tetrapyrroles such as **13**, and the eudistomins (*e.g.* eudistomodin A **62a**, eudistomin B **109**, and eudistomin C **62b**). Eudistomins have the same coordinating groups as the iron(II) chelator pyrimine isolated from a *Pseudomonas* species.[276] The peptides and 3,4,5-trihydroxyphenyl groups are both found in celenamide E **110**, and in tunichromes **32a** and **32b**.

Adenochromines **26a–c** have a particularly interesting array of donor groups with catechol, thioether, imidazole and amino acid groupings.[276] The thiazole ring is found in a range of aza-crown cyclic compounds, and the oxazolone ring in ulicyclamide and lissoclinamides. Other pigments such as 2-bromoleptoclinidinone **38**, ascididemin **43**, tjipanazoles D **111** and J **112**, shermilamines A and B **40a+b**, nordercitin **113**, kuanoniamines A **37** and D **114**, plankinidine D **45**, anguibactin **115**, dysidenin **116**, pyrimine **117**, clionamide **118**, and bisimidazoles such as paragracin **119**, all have the potential to act as ligands.[275,276]

106

107

108

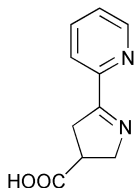**109**

110

111

112

113

114
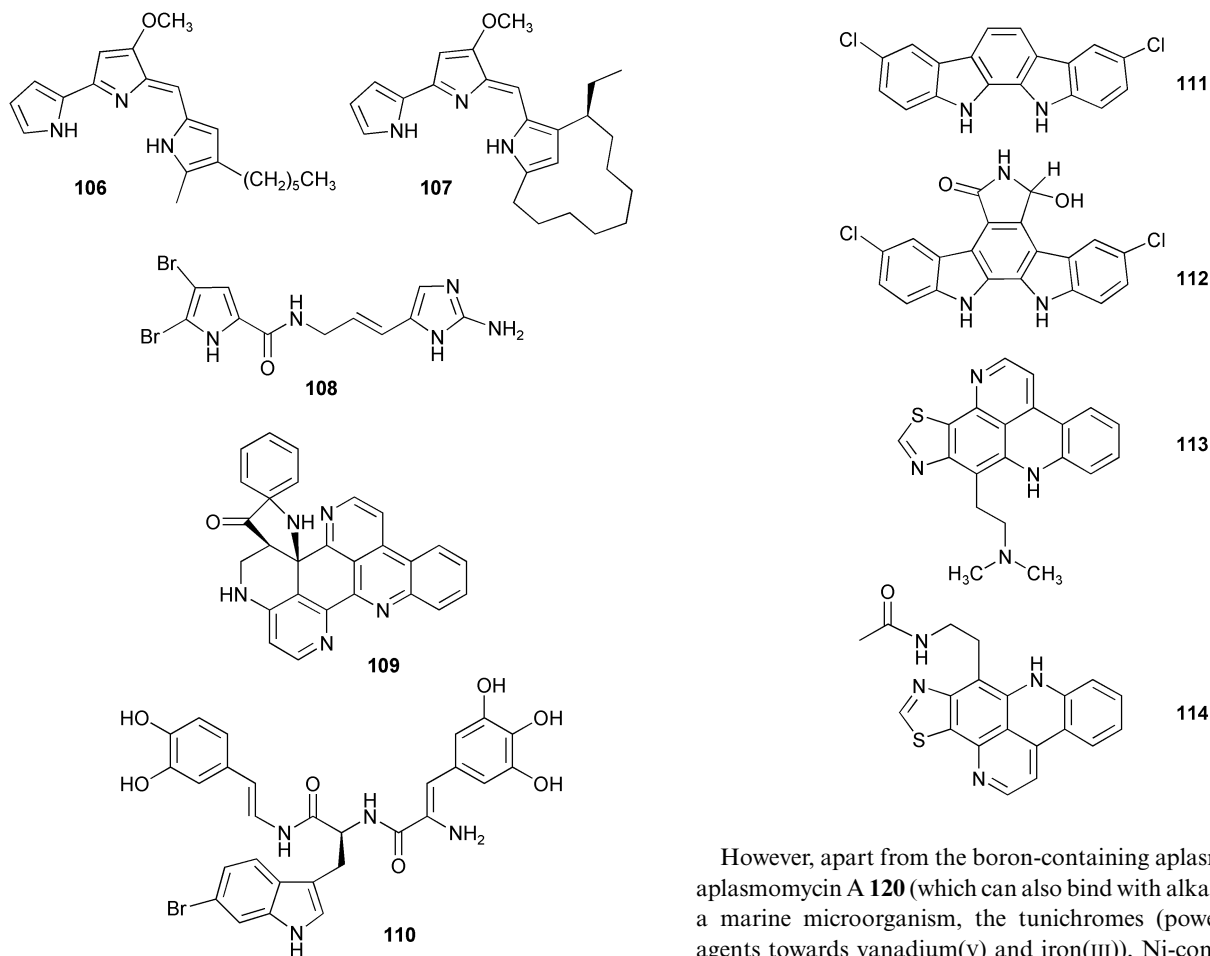
However, apart from the boron-containing aplasmomycins, *e.g.* aplasmomycin A **120** (which can also bind with alkali metals) from a marine microorganism, the tunichromes (powerful reducing agents towards vanadium(V) and iron(III)), Ni-containing eilatin
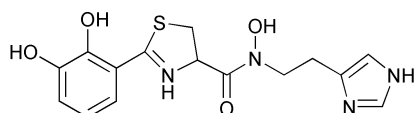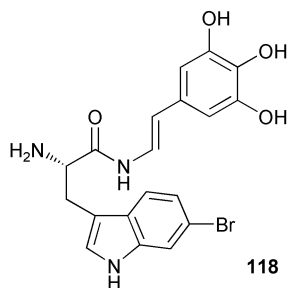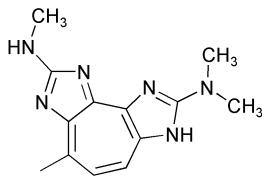
115
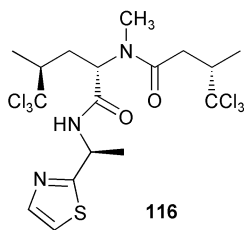
116

117

118

119

39, the blue-green pigment tunichlorin **121**, the guanidine alkaloid clathridine **122** together with its zinc complex, geodiastatins A and B (brownish-black silicon-containing luminescent chromoproteins from the sponge *Geodia mesotriaena*), the iron protein from the blood cells of the stolidobranch *Pyura stolonifera*,[275,276] and the ubiquitous magnesium-containing chlorophylls, examples of metal-containing complexes are rare.
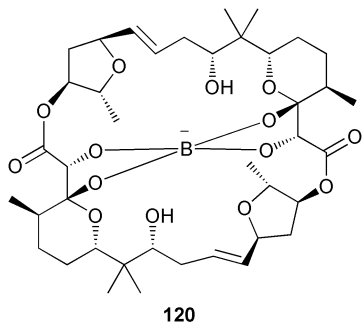

120
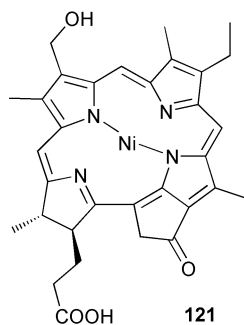
**121**
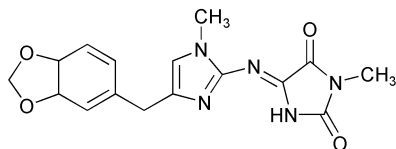
122

In contrast, a large number of metal-free tetrapyrrole metabolites, including the pigments chlorophyllone A, bonellin **123**, corallistins A–E **124a–e**, and cyclopheophorbide enol **125** have been characterised. The cyclic peptide jasplakinolide (jaspamide) **126** from *Jaspis johnstoni*, corallistine **101**, naamidine A **102a**, isonaamidine A **102b** and pyronaamidine **127**, structurally similar to clathridine (but devoid of the metal), have been identified from different sponges.[89,90,275–277]

These compounds are certainly capable of forming metal complexes. The apo-protein, ferreascidin, derived from the iron protein from the blood cells of the stolidobranch *Pyura stolonifera*,[276] complexes with iron to produce a blue pigment. The violet adenochromes, Fe^{3+}-binding pigments from *Octopus vulgaris*, on removal of the Fe^{3+} ion, produce the colourless adenochromines **26a–c**. These colourless desferri-adenochromes react with iron(III) chloride to form a blue precipitate. Both reactions appear to be pH-dependent, the reaction taking place at pH 4.5.[34,114] It is well known that sea squirts assimilate metals such as vanadium, manganese, nickel and iron from sea-water. For example, the tunicate *Ascidia nigra* accumulates vanadium to produce yellow pigments, the tunichromes, whereas *Trididemnum solidum* produces the nickel-containing blue-green pigment tunichlorin[278] **121**, and *Molgula manhattensis* sequesters iron.[275] The Papua New Guinean shell-less mollusc *Dolabella auricularia* contains a series of green to blue-green chlorins (2,3-dihydroporphyrins) **11**, one of which was identified as the nickel chelate tunichlorin, previously isolated from *T. solidum*.[278] Ascidians accumulate vanadium ions (up to 1 M) from sea-water, where vanadium is present in the +5 oxidation state. In ascidian blood cells, however, the vanadium was found to be in the +3 and/or +4 states. In *Ascidia nigra*, at least 90% of the total vanadium is in the oxygen-sensitive +3 state. Some adenochromines from *Ascidia nigra*[279] and *T. solidum*[278] showed vanadium(III) (green) and vanadium(IV) (blue) oxidation levels. These oxygen-sensitive pigments underwent pH-dependent reactions with vanadium(IV) and vanadium(V) to produce insoluble green or green-black polynuclear complexes. During this process vanadium vanadium(V) and vanadium(IV) were reduced to the +3 oxidation level.[280,281] Eilatin **39**, a 1,10-phenanthroline derivative, complexes with nickel, ruthenium and osmium. The nickel complex is blue, but the ruthenium complexes are green or red, depending on the ratio of ruthenium to eilatin. The fused-ring, pentacyclic, biologically active alkaloid 2-bromoleptoclinidone **111**, isolated from the Mediterranean ascidian *Cystodytes dellechiajei*, possesses a bidentate chelating site which forms a complex with ruthenium(II).[275,282–284] Derivatives of patellamide A **128** reacts with copper(II) to yield compounds absorbing at wavelengths of 675 nm and 687 nm.[286] The acyl tetramic acid, discodermide **129**, a macrocyclic lactam from the deep-water sponges *Discodermia dissoluta*[285] and *D. polydiscus*[277] forms an orange-red complex with iron(III) chloride and a greenish-yellow complex with copper(III) acetate.[275,285] The removal of Fe^{3+} results in loss of the absorption maximum with the formation of a pale yellow substance, which is capable of binding with Fe(III) and regenerating the original pigment. The known naamidines, novel zinc complexes of naamidine-type alkaloids A and G, and the first natural marine mixed-ligand zinc(II) metal complex **130** have been characterised from a calcareous sponge, *Leucetta* sp., of the Coral Sea. It is noteworthy that complexing with zinc did not bring about significant changes in the absorption maxima of the parent compounds, usually observed with other metabolite–metal chelates. The absorption maxima of the naamidines (λ_{max} 385 nm), bis(naamidinato)zinc(II) complexes A:A (λ_{max} 380 nm), G:G (λ_{max} 386 nm), and A:G (λ_{max} 390 and 373 nm) were very similar.[287] In addition, the animals were snow-white to faintly brown in appearance.

Apart from common metal cations such as calcium, magnesium, potassium and sodium, (concentrations below 10^3 M), most other

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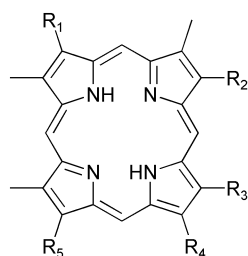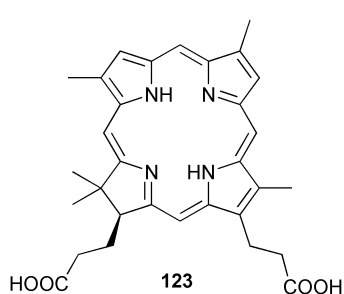
	R_1	R_2	R_3	R_4	R_5
124a	H	C_2H_5	$CH_2CO_2CH_3$	$(CH_2)_2CO_2CH_3$	C_2H_5
124b	C_2H_5	C_2H_5	$CH_2CO_2CH_3$	$(CH_2)_2CO_2CH_3$	C_2H_5
124c	C_2H_5	H	CH_3	$(CH_2)_2CO_2CH_3$	$(CH_2)_2CO_2CH_3$
124d	CH_3	H	CH_3	$(CH_2)_2CO_2CH_3$	$(CH_2)_2CO_2CH_3$
124e	H	H	CH_3	C_2H_5	$(CH_2)_2CO_2CH_3$

123

125

126

127

128

129

130

naamidinato A

naamidinato G
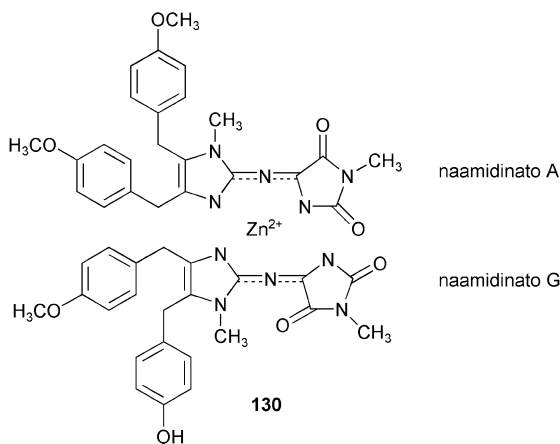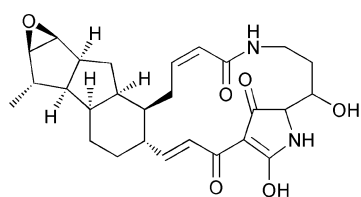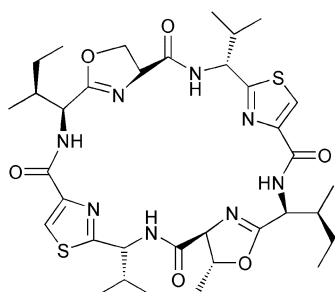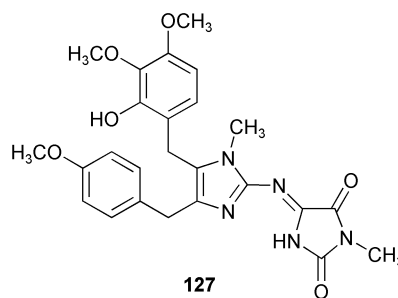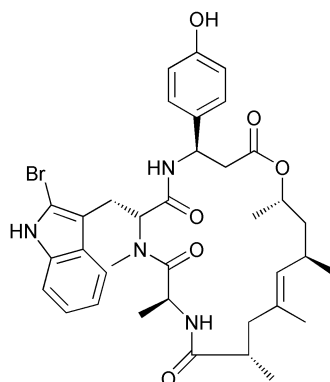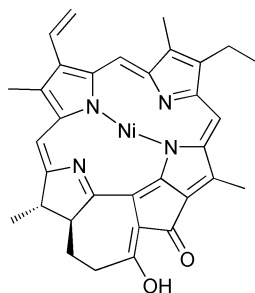
metal ions such as copper, cadmium, cobalt, iron, manganese, molybdenum, nickel, vanadium and zinc occur in concentrations below 10^{-4}–10^{-6} M in sea-water.[292] The metabolic cost of accumulating these ions selectively implies some role in the organisms, the elucidation of which remains a challenge. For most of the above chelates, their roles and the importance of metals in these roles are not known. So for what purpose are these secondary metabolites produced by marine organisms, and why do some of them accumulate metal ions present in such low concentrations? Several hypotheses have been proposed. Among them are the speculations that they are involved in the uptake and transport of metal ions, that they have a role to play in the biological activity of these metabolites, that the metal chelates provide a template for biological assembly of the metabolites, and that they maintain the solubility of concentrated vanadium at biological pH values.[275,276,280]

All organisms require oxygen in order to derive compounds that yield energy, and they have adapted many mechanisms to cope with changing external oxygen supply. One such mechanism is the use of pigments such as hemocyanin, hemoerythrin, chlorocruorin and haemoglobin to bind molecular oxygen and deliver it to cells. Chlorocruorin is the chemical substance that is supposed to cause the green colour of the blood of certain marine polychaete annelids. It is an extracellular respiratory pigment, and binds one Fe^{2+} ion with one molecule of oxygen – it is similar to haemoglobin due to the fact that it too has a heme group. The nickel chelate tunichlorin, found in the sea hare *Dolabella auricularia* (the same blue-green pigment found in the tunicate *Trididemnum solidum*, which has variable colouration), may play an important role in electron transfer or other metabolic processes.[293]

The metabolites that form or have the potential to form complexes with metals (*e.g.* i–viii and x–xxv, Table 1) usually absorb in the far-UV-B and near-UV-A regions. There is sufficient evidence to postulate that chelation is accompanied by a bathochromic shift of the absorption maxima to the visible region. For example, the UV-VIS absorption spectrum of a porphyrin exhibits an intense

peak at about 420 nm followed by several weak peaks at longer wavelengths (from 500–650 nm). Reduction of one or more pyrrole rings and chelation with magnesium (as in chlorophyll-a) results in a strong absorption peak at approximately 650 nm.[294] The yellow pigment tunichrome from the ascidian *Ascidia nigra* produces an insoluble blue or green-black polymer complex with vanadium (indeed, some of these animals are blue or black in colour).

The colours of the corresponding organisms varied from brilliant yellow to bright green and blue, shades of violet – some are even black (Table 1). (However, these descriptive colours should be treated with caution, for marine invertebrates show variations in colour and morphology relative to the light regime of the habitat, depth, and the geographical location, *etc.*[178]). Is it then reasonable to postulate that the chelation of these "ligands" with metals brings about changes in the colour of the pigments (at least in some of the animals), that the colour of the organism is a reflection of the colour of the pigment–metal chelate, and that the chelation is destroyed during the extraction/purification process?

15   Conclusion

The key to the existence of a species is its ability to survive long enough to successfully reproduce and pass its genes on to the next generation. During the evolution of animals, most of them developed defence mechanisms to improve their chances of surviving in a changing world. The accurate knowledge of factors affecting the survival of early life stages of marine invertebrates is critically important for the understanding of their population dynamics and the evolution of their diverse reproductive and life history characteristics. Chemical defence is an important determinant of survival for larvae, juvenile as well as adult stages of many sessile benthic invertebrates, yet relatively little consideration has been given to chemical defences at the early life stages. Information on the localisation of defensive secondary metabolites in larvae and the fitness-related consequences of consuming even a small amount of toxic secondary metabolite underpin proposals regarding the potential for Mullerian and Batesian mimicry to occur among marine larvae.[279]

Coral reefs are among the oldest and largest living entities on earth, whose individual components live or die together, rather like a forest. Vast numbers of creatures live in them, including an estimated quarter of the world's oceanic fish.[295] There are many amazing strategies, adaptations, and behaviours that marine plants and animals have developed in order to be able to live and flourish in our greatest ecosystem, the ocean. From microscopic plants and animals to some of the ocean's behemoths such as the giant manta ray, these organisms reveal how each one fits into its own special niche. The violent cuttlefish mating ritual ensures that only the strongest will reproduce. Sea anemones might look beautiful to us but not to a shrimp captured by thousands of their miniature harpoons, injected with poison and slowly devoured. Sea hares might appear dull in comparison to their fellow sea slugs, the more flamboyant nudibranchs, but they can make huge mating chains, with each individual acting as male and female to different neighbours. Sea hares live no more than a year, but what a year! Females of the humble-looking slipper limpet secrete a substance into the water that maintains the masculinity of nearby males. As on land, the shape of a plant or animal will determine how and where they fit into the larger picture. In the ocean, as on land, some creatures are built for speed, while others seldom move, or are in fact sessile. Others are still at the mercy of wind and current for transportation, and this exquisitely complex ecosystem is very vulnerable to many environmental factors.

Our knowledge about the role and importance of colouration in marine organisms is in its infancy. The colour characteristics of organisms serve various purposes and are the result of several different processes. It is likely that there is no one single reason why marine invertebrates are coloured. It may be that the colours could be clues to physiological processes, developmental conditions, environmental interactions *etc.*, or a combination of these factors. The number of alkaloidal pigments from marine organisms mentioned in this article is only a "drop in the ocean" compared with what is reported in the literature.[74,113,126,236,239,261,275,277,296–300] These organisms produce a wide array of nitrogenous compounds with diverse structures, yet very little is known of their roles in these organisms.

Mimicry is a great example of evolution by natural selection, and has been celebrated for this ever since. But there are still many puzzling aspects of mimicry that need to be studied – for example, the molecular nature of the genes involved, and whether random drift may initiate some of these changes. Carefully controlled field and laboratory studies on aposematism are needed before general inferences can be adequately evaluated. Nevertheless, the examples that are presently known are intriguing and strongly suggestive that crypsis, aposematic colouration (both Batesian and Mullerian), are effective defensive strategies in the marine environment. In order to assess the functional role of pigments, bioassays that model ecological interactions, such as predator–prey and other survival-related relationships, must be employed. Opisthobranch molluscs, by virtue of the fact that they contain diverse and widespread toxins and exhibit an array of distinct and vibrant colour patterns, are ideal organisms for further study and experimentation. Studies on adaptive colouration among invertebrates could show important differences in protective mechanisms between and within taxa as well as between land, freshwater and marine habitats. With the coupling of innovative analytical techniques with physical methods (such as nuclear magnetic resonance, mass spectrometry and X-ray crystallography with tools of modern genetics and mechanistic biology) put to the service of identifying complex pigments and metabolites, preparing and isolating pigment–metal complexes, finding evidence for metal–metabolite interactions and carrying out adaptive studies of colour pattern variation,[296] we can look forward to a new era of understanding of this venerable aspect of evolutionary biology.

16   References

1 G. Y. Kennedy, *Adv. Mar. Biol.*, 1979, **16**, 309.
2 J. E. N. Veron, *Corals of Australia and the Indo-Pacific*, University of Hawaii Press, Honolulu, 1986.
3 J. E. N. Veron, *Corals of the World*, vols. 1–3, ed. M. Stafford-Smith, Australian Institute of Marine Science, Queensland, Australia, 2000.
4 H. Rossotti, *Colour*, Princeton University Press, Princeton, New Jersey, 1985.
5 D. L. Fox, *Comp. Biochem. Physiol., B*, 1976, **55**, 137.
6 D. L. Fox, *Animal biochromes and structural colors* (2nd edn), University of California Press, Berkeley, 1976.
7 A. E. Needham, *The significance of zoochromes*, Springer-Verlag, New York, 1974..

This journal is © The Royal Society of Chemistry 2006

Nat. Prod. Rep., 2006, **23**, 223–255 | 251

8 V. Pearse, J. Pearse, M. Buchsbaum and R. Buchsbaum, *Living invertebrates*, Blackwell Science Publications, Palo Alto, California, 1987.

9 T. W. Goodwin, *The Comparative Biochemistry of the Carotenoids*, Chapman and Hall, London, 1952.

10 T. W. Goodwin, in *Chemical Zoology*, ed. M. Florkin and B. T. Scheer, Academic Press, New York/London, 1968, vol. 11, p. 149.

11 T. W. Goodwin, in *Chemical Zoology*, ed. M. Florkin and B. T. Scheer, Academic Press, New York/London, 1972, ch. 8.

12 G. R. Pettit, J. A. Rideout and J. A. Hasler, *J. Nat. Prod.*, 1981, **44**, 588.

13 J. L. Sumich, in *An Introduction to the Biology of Marine Life*, ed. J. L. Sumich and I. Dubuque, Wm. C. Brown Publishers, 1980, p. 126.

14 I. V. Kelmanson and M. V. Matz, *Mol. Biol. Evol.*, 2003, **20**, 1133.

15 P. G. Falkowski and Z. Dubinsky, *Nature*, 1981, **289**, 172.

16 R. J. Blank and R. K. Trench, *Science*, 1985, **229**, 656.

17 K. Rutzler and I. G. Macintyre, *Contrib. Mar. Sci.*, 1982, **12**, 9.

18 T. A. Stephenson, *Endeavour*, 1947, **6**, 152.

19 V. J. Paul and M. P. Puglisi, *Nat. Prod. Rep.*, 2004, **21**, 189.

20 A. Kelecom, *An. Acad. Bras. Cienc.*, 2002, **74**, 1.

21 M. K. Wicksten, in 'Adaptive coloration in invertebrates', *Proc. Symp. Am. Soc. Zool.*, ed. M. K. Wicksten, Galveston, TX, USA, 1990, vol. 127.

22 W. M. Bandaranayake and A. Des Rocher, *Mar. Biol.*, 1999, **133**, 163.

23 W. M. Bandaranayake, *Nat. Prod. Rep.*, 1998, **15**, 159.

24 W. M. Bandaranayake and W. A. Wickramasinghe, *Comp. Biochem. Physiol., B*, 1996, **113**, 499.

25 W. M. Bandaranayake, *Trends Comp. Biochem. Physiol.*, 2002, **9**, 229.

26 J. B. McClintock, B. J. Baker and D. K. Steinberg, in *Marine Chemical Ecology*, ed. J. B. McClintock and B. J. Baker, CRC Press, New York, 2001, p. 195.

27 D. L. Fox and C. F. A. Pantin, *Biol. Rev.*, 1944, **19**, 121.

28 P. Proksch, *Toxicon*, 1994, **32**, 639.

29 P. R. Bergquist, *Sponges*, Hutchinson and Co., London, 1978.

30 R. H. Thompson, *Chem. Br.*, 1978, **14**, 133.

31 R. A. de Reaumur, *Mem. Acad. R. Sci.*, 1771, 118.

32 P. Friedlander, *Monatsh. Chem.*, 1907, **28**, 991.

33 P. Friedlander, *Ber. Dtsch. Chem. Ges.*, 1909, **42**, 765.

34 G. Prota, in *Marine Natural Products: Chemical and Biological Perspectives*, ed. P. J. Scheuer, Academic Press, New York, 1980, p. 141.

35 T. Goto, in *Marine Natural Products: Chemical and Biological Perspectives*, ed. P. Scheuer, Academic Press, New York, 1980, p. 179.

36 M. Fontaine, *C. R. Hebd. Seances Acad. Sci.*, 1934, **180**, 1369.

37 V. Tartar, *The Biology of Stentor*, Pergamon Press, Oxford, 1961.

38 A. C. Giese and R. M. Grainger, *Photochem. Photobiol.*, 1970, **12**, 489.

39 E. Faure-Fremiet, *Biol. Bull. (Woods Hole, MA, U. S.)*, 1936, **70**, 353.

40 A. R. Fontane, *J. Mar. Biol. Assoc. U. K.*, 1962, **42**, 9.

41 C. A. MacMunn, *Q. J. Microsc. Sci.*, 1890, **30**, 51; C. A. MacMunn, *J. Physiol.*, 1899, **24**, 1.

42 M. I. Newbigin, *Color in Nature*, John Murray, London, 1898.

43 A. Abeloos-Parize and R. Abeloos-Parize, *C. R. Seances Soc. Biol. Ses. Fil.*, 1926, **94**, 560.

44 J. Verne, *Couleurs et Pigments des etres vivants*, Armand Colin, Paris, 1930.

45 E. Lederer, *C. R. Seances Soc. Biol. Ses. Fil.*, 1933, **113**, 1391.

46 D. F. Cheesman, W. L. Lee and P. F. Zagalsky, *Biol. Rev.*, 1967, **42**, 132.

47 B. C. L. Weedon, in *Carotenoids: Occurrence of Carotenoids*, ed. O. Osler, Birkhauser Verlag, Basel/Stuttgart, 1971.

48 G. Y. Kennedy, in *Chemical Zoology*, ed. M. Florkin and B. T. Scheer, Academic Press, New York, 1969, ch. 10.

49 A. Comfort, *Biol. Rev.*, 1951, **26**, 285.

50 D. L. Fox and H. G. Vevers, *The Nature of Animal Colors*, Sidgwick & Jackson, London, 1960.

51 B. M. Gilchrist, *Comp. Biochem. Physiol.*, 1968, **24**, 123.

52 D. L. Fox, in *Biochemical and Biophysical Perspectives in Marine Biology*, ed. J. R. Sargent and D. Malins, Academic Press, New York/London, 1974, p. 1.

53 C. F. W. Krukenberg, *Die Pigmente: ihre Eigenschaften, ihre Genese und ihre Metamorphosen bei wirbellosen Tieren*, Vergleichende physiologische Studien, Heidelberg, 1882, p. 92.

54 P. J. Herring, *Comp. Biochem. Physiol., B*, 1971, **39**, 739.

55 P. F. Zagalsky, F. Haxo, S. Hertzberg and S. Liaaen-Jensen, *Comp. Biochem. Physiol., B*, 1989, **93**, 339.

56 I. M. Heilbron, H. Jackson and R. N. Jones, *Biochem. J.*, 1935, **29**, 1384.

57 D. L. Fox and C. F. A. Pantin, *Philos. Trans. R. Soc. London, Ser. B*, 1941, **230**, 415.

58 J. Roche, *Bull. Soc. Chim. Biol.*, 1936, **18**, 825.

59 H. N. Moseley, *Q. J. Microsc. Sci.*, 1877, **17**, 1.

60 P. J. Herring, *Nature*, 1972, **238**, 276.

61 G. Y. Kennedy and H. G. Vevers, *Comp. Biochem. Physiol., B*, 1976, **55**, 117.

62 H. C. Sorby, *Q. J. Microsc. Sci.*, 1875, **15**, 166.

63 A. Pelter, J. A. Ballantine, V. Ferrito, V. Jaccarini, A. P. Psaila and P. J. Schembri, *J. Chem. Soc., Chem. Commun.*, 1976, 999.

64 J. Cote, *C. R. Seances Soc. Biol. Ses. Fil.*, 1903, **55**, 137.

65 D. L. Fox and N. Millott, *Proc. R. Soc. London, Ser. B*, 1954, **142**, 392.

66 R. Tixier, *Ann. Inst. Oceanogr. (Monaco)*, 1945, **22**, 343.

67 M. Abeloos and G. Teissier, *Bull. Soc. Zool. Fr.*, 1926, **51**, 145.

68 E. Lederer, *Biol. Rev.*, 1940, **15**, 273.

69 G. Prota, S. Ito and G. Nardi, in *Marine Natural Products Chemistry*, ed. J. Faulkner and W. H. Fenical, Plenum, New York, 1977, p. 45.

70 E. Beerstechner, *Vitam. Horm.*, 1950, **10**, 69.

71 S. Yamamura and Y. Hirata, *Tetrahedron*, 1963, **19**, 1485.

72 K. D. Barrow, in *Marine Natural Products*, ed. P. J. Scheuer, 1983, p. 51.

73 M. J. Garson, in *Marine Chemical Ecology*, ed. J. B. McClintock and B. J. Baker, CRC Press, Boca Raton/London/New York, 2001, p. 71.

74 D. J. Faulkner, *Nat. Prod. Rep.*, 1991, **8**, 97 (and references cited therein).

75 O. F. Smetanina, L. S. Schevchenko, T. A. Kuznetsova, V. V. Mikhailov and G. B. Elyakov, in 'Bridges of Science between North America and Russian Far East', *45th Arctic Science Conference*, Anchorage, AK, USA, 1994, paper no. 299.

76 J. Shin, Y. Seo, K. W. Cho, J. R. Rho and C. J. Sim, *J. Nat. Prod.*, 1999, **62**, 647.

77 T. W. Goodwin, *The Biochemistry of Carotenoids*, Chapman Hall, New York, 1980, vol. 2.

78 H. Pfander, *Key to Carotenoids*, Birkhauser, Basel, 1987.

79 S. Liaaen-Jensen, *New J. Chem.*, 1990, **14**, 747.

80 T. Matsuno, M. Ookubo, T. Nishizawa and I. Shimizu, *Chem. Pharm. Bull.*, 1984, **32**, 4309.

81 T. Matsuno and M. Tsushima, *Comp. Biochem. Physiol., B*, 1995, **111**, 597.

82 A. Kitamura, J. Tanaka and T. Higa, *J. Nat. Toxins*, 1996, **5**, 219.

83 P. S. Parameswaran and C. T. Achuthankutty, *Proc. Andhra Pradesh Akad. Sci.*, 2005, **9**, 135.

84 T. Bjornland, A. Fiksdahl, T. Skjetne, J. Krane and S. Liaaen-Jensen, *Tetrahedron*, 2000, **56**, 9047.

85 M. H. Gorden, *Nat. Prod. Rep.*, 1996, **13**, 265.

86 T. Kawakami, M. Tsushima, Y. Katambi, M. Mine, A. Ishida and T. Matsuno, *J. Exp. Mar. Biol. Ecol.*, 1998, **226**, 165.

87 G. Y. Kennedy, *Ann. N. Y. Acad. Sci.*, 1975, **244**, 662.

88 P. Karuso and P. J. Scheuer, *Molecules*, 2002, **7**, 1.

89 M. D'Ambrosio, A. Guerriero, C. Debitus, O. Ribes, B. Richer de Forges and F. Pietra, *Helv. Chim. Acta*, 1989, **72**, 1451.

90 M. D'Ambrosio, A. Guerriero, C. Debitus, O. Ribes and F. Pietra, *Helv. Chim. Acta*, 1993, **76**, 1489.

91 R. A. Nicolaus, *Melanins*, Hermann, Paris, 1968.

92 R. A. Nicolaus, in *Methodicum chimicum (Part 3)*, ed. F. Korte, Academic Press, New York, 1978, p. 190.

93 G. Prota, *Med. Res. Rev.*, 1988, 8.

94 D. L. Fox and T. S. Hopkins, in *Physiology of Echinodermata*, ed. R. A. Boolootian, Interscience Publishers, New York, 1966, p. 277.

95 H. S. Mason, in *Pigment cell growth*, ed. M. Gordon, Academic Press, New York, 1953, p. 207.

96 M. Piattelli and R. A. Nicolaus, *Tetrahedron*, 1961, **15**, 66.

97 R. H. Thomson, *Naturally Occurring Quinones*, Chapman and Hall, London, 1987.

98 I. R. Smith and M. D. Sutherland, *Aust. J. Chem.*, 1971, **24**, 1487.

99 G. Cimino, S. De Stefano and L. Minale, *Comp. Biochem. Physiol., B*, 1974, **47**, 895.

100 H. Singh, R. E. Moore and P. G. Scheuer, *Experientia*, 1967, **23**, 624.

101 K. A. Francesconi, *Aust. J. Chem.*, 1980, **33**, 2781.

102 J. A. Rideout and M. D. Sutherland, *Aust. J. Chem.*, 1985, **38**, 793.

103 J. A. Rideout, N. B. Smith and M. D. Sutherland, *Experientia*, 1979, **35**, 1273.
104 D. M. Balitz, J. A. Bush, J. A. and W. T. Bradner, *J. Antibiot.*, 1982, **35**, 259.
105 G. Cimino and G. Sodano, in *Sponges in Space and Time*, ed. R. W. M. Van Soest, T. M. G. Van Kempen and J. S. Breakman, Balkema, Rotterdam, 1994, p. 459.
106 L. Cariello, M. De Nicola Giudici and L. Zanetti, *Comp. Biochem. Physiol., C*, 1980, **65**, 37.
107 Y. C. Shen, C. H. Lu, R. Chakraborty and Y. H. Kuo, *Nat. Prod. Res.*, 2003, **17**, 83.
108 G. Cimino, S. De Rosa and S. De Stefano, *Experientia*, 1982, **38**, 896.
109 D. M. Roll, P. J. Scheuer, G. K. Matsumoto and J. Clardy, *J. Am. Chem. Soc.*, 1983, **105**, 6177.
110 T. Higa, T. Fujiyama and P. J. Scheuer, *Comp. Biochem. Physiol., B*, 1980, **65**, 525.
111 C. J. Smith, R. L. Hettich, J. Jompa, A. Tahir, M. V. Buchanan and C. M. Ireland, *J. Org. Chem.*, 1998, **63**, 4147.
112 J. A. Rideout and M. D. Sutherland, *Aust. J. Chem.*, 1981, **34**, 2385.
113 D. Laurent and F. Pietra, *Chem. Biodiversity*, 2004, **1**, 539.
114 C. Christophersen and U. Anthoni, *Sulfur Rep.*, 1986, **4**, 365 (and references cited therein).
115 Y. Seo, J. R. Rho, N. Geum, J. B. Yoon and J. Shin, *J. Nat. Prod.*, 1996, **59**, 985.
116 D. A. Webb, *J. Exp. Biol.*, 1939, **16**, 499.
117 I. G. Macara, G. C. McLeod and K. Kustin, *Biochem. J.*, 1979, **181**, 457.
118 C. Christophersen, in *Marine Natural Products: Chemical and Biological Perspectives*, ed. P. J. Scheuer, Academic Press, New York, 1983, p. 259.
119 J. T. Baker, *Endeavour*, 1974, **33**, 11.
120 L. Carriello, S. Crescenzi, G. Prota, F. Giordano and L. Mazzarella, *J. Chem. Soc., Chem. Commun.*, 1973, 99.
121 L. Carriello, S. Crescenzi, G. Prota and L. Zanetti, *Tetrahedron*, 1974, **30**, 4191.
122 L. Carriello, M. De Nicola-Giudici, L. Zanetti and G. Prota, *Proc. Congr. Soc. Ital. Biol. Mar.*, 1977, **9**, 171.
123 L. Carriello, M. De Nicola-Giudici, S. Crescenzi and G. Prota, *Experientia*, 1978, **34**, 1460.
124 C. Jimenez and P. Crews, *J. Nat. Prod.*, 1993, **56**, 9.
125 H. Hoffmann and T. Lindel, *Synthesis*, 2003, 1753.
126 D. J. Faulkner, *Nat. Prod. Rep.*, 2002, **19**, 1 (and references cited therein).
127 T. F. Molinski, *Chem. Rev.*, 1993, **93**, 1825.
128 K. M. Marshall and L. R. Barrows, *Nat. Prod. Rep.*, 2004, **21**, 731.
129 A. R. Carrol, B. F. Bowden and J. C. Coll, *Aust. J. Chem.*, 1993, **46**, 489.
130 S. Urban, L. Hobbs, J. N. A. Hooper and R. J. Capon, *Aust. J. Chem.*, 1995, **48**, 1491.
131 P. W. Ford and B. S. Davidson, *J. Nat. Prod.*, 1997, **60**, 1051.
132 H. C. Vervoort, W. Fenical and P. A. Keifer, *J. Nat. Prod.*, 1999, **62**, 389.
133 M. Gompel, M. Leost, E. B. De, K. Joffe, L. Puricelli, L. H. Franco, J. Palermo and L. Meijer, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 1703.
134 W. M. Bandaranayake, *AIMS Rep.*, no. 18, 1994.
135 H. W. Lam, P. A. Cooke, G. Pattenden, W. M. Bandaranayake and W. Wickramasinghe, *J. Chem. Soc., Perkin Trans.*, 1999, 847.
136 W. M. Bandaranayake, G. Pattenden and W. A. Wickramasinghe, *Trends Comp. Biochem. Physiol.*, 2002, **9**, 205.
137 A. Zampella, M. V. D'Auria, C. Debitus and J. L. Menou, *J. Nat. Prod.*, 2000, **63**, 943.
138 J. H. Kwak, F. J. Schmitz and M. Kelly, *J. Nat. Prod.*, 2000, **63**, 1153.
139 A. Marin, M. D. Lopez Belluga, G. Scognamiglio and G. Cimino, *J. Molluscan Stud.*, 1997, **63**, 431.
140 C. Jimenez, E. Quinoa and M. Adamczeski, *J. Org. Chem.*, 1991, **56**, 3403.
141 T. Shindo, A. Sato, N. Kasanuki, K. Hasegawa, S. Sato, T. Iwata and T. Hata, *Experientia*, 1993, **49**, 177.
142 S. H. D. Haddock, C. W. Dunn, P. R. Pugh and C. E. Schnitzler, *Science*, 2005, **309**, 263 (and references cited therein).
143 B. H. Robinson, *J. Mar. Biol. Assoc. U. K.*, 1992, **72**, 463.
144 A. K. Campbell and P. J. Herring, *Mar. Biol.*, 1990, **104**, 219.
145 F. H. Johnson and O. Shimomura, in *Methods in Enzymology*, ed. M. A. DeLuca, Academic Press, New York, 1978, vol. LVII, p. 331.
146 A. Salih, A. Larkum, G. Cox, M. Kuhl and O. Hoegh-Guldberg, *Nature*, 2000, **408**, 850.
147 C. H. Mazel, T. W. Cronin, R. L. Caldwell and N. J. Marshall, *Science*, 2004, **303**, 51.
148 K. Edlinger, *Malacologia*, 1982, **22**, 1.
149 I. Bravo, J. M. Franco, A. Alonso, R. Dietrich and P. Molist, *Mar. Biol.*, 2001, **138**, 709.
150 M. Tsuda, D. Sakurai and M. Goda, *J. Exp. Biol.*, 2003, **206**, 1409.
151 H. Takata and T. Kominami, *Zool. Sci.*, 2002, **19**, 1450.
152 K. L. Gosner, *A field guide to the Atlantic seashore*, Houghton Mifflin, Boston, 1979.
153 E. C. Haderlie, C. Hand and W. G. Gladfelter, in *Intertidal invertebrates of California*, ed. R. H. Morris, D. P. Abbott and E. C. Haderlie, Stanford University Press, Stanford, 1980, p. 40.
154 G. J. Bakus and D. P. Abbott, in *Intertidal invertebrates of California*, ed. R. H. Morris, D. P. Abbott and E. C. Haderlie, Stanford University Press, Stanford, 1980, p. 21.
155 M. Kimura, *Sci. Am.*, 1979, **24**, 98.
156 E. Savazzi, *Lethaia*, 1998, **31**, 15.
157 F. Garcia-Pichel, N. D. Sherry and R. W. Castenholz, *Appl. Environ. Microbiol.*, 1993, **59**, 170.
158 C. S. Cockell and J. Knowland, *Biol. Rev.*, 1999, **74**, 311.
159 D. Karentz, in *Marine Chemical Ecology*, ed. J. B. McClintock and B. J. Baker, CRC Press, New York, 2001, p. 481.
160 W. C. Dunlap and J. M. Shick, *J. Phycol.*, 1998, **34**, 418.
161 C. Nageli and S. Schwenderer, *Das Mikroskop*, W. Englemann, Leipzig, 1877.
162 F. Garcia-Pichel and R. W. Castenholz, *J. Phycol.*, 1991, **27**, 395.
163 F. Garcia-Pichel, F. Wingard and R. W. Castenholz, *Photochem. Photobiol.*, 1992, **56**, 17.
164 P. J. Proteau, W. H. Gerwick, F. Garcia-Pichel and R. W. Castenholz, *Experientia*, 1993, **49**, 825.
165 J. W. Porter and N. M. Targett, *Biol. Bull.*, 1988, **175**, 230.
166 N. I. Krinsky, *Pure Appl. Chem.*, 1979, **51**, 649.
167 A. J. Young, *Physiol. Plant.*, 1991, **83**, 702.
168 B. Demmig-Adams and W. W. Adams, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1992, **43**, 599.
169 A. D. Patil, A. J. Freyer, L. Killmer, G. Hofmann and R. K. Johnson, *Nat. Prod. Lett.*, 1997, **9**, 201.
170 I. Kitagawa, M. Kobayashi, K. Kitanaka, M. Kido and Y. Kyogoku, *Chem. Pharm. Bull.*, 1983, **31**, 2321.
171 N. Lindquist, E. Lobkovsky and J. Clardy, *Tetrahedron Lett.*, 1996, 9131.
172 N. L. Lindquist, *US Pat. 5705146*, 1998.
173 N. Lindquist, M. E. Hay and W. Fenical, *Ecol. Monogr.*, 1992, **62**, 547.
174 W. M. Bandaranayake, 2005, unpublished results.
175 R. Riedl, *Eine Monographie*, Paul Farey, Hamburg, 1966.
176 G. L. Voss, *Seashore life of Florida and the Caribbean*, E. A. Seemann, Miami, Florida, 1976.
177 E. A. Titlyanov, M. G. Shaposhnikova and V. I. Zvalinsky, *Photosynthetica*, 1980, **14**, 413.
178 R. R. Olson, *Biol. Bull.*, 1986, **170**, 62.
179 D. J. Barnes and B. E. Chalker, in *Ecosystems of the World: Coral Reefs*, ed. Z. Dubinsky, Elsevier, New York, 1990, p. 109.
180 B. E. Brown, in *Advances in Marine biology*, ed. J. H. S. Blaxter and A. J. Southward, Academic Press, London/Sydney/New York, 1997, vol. 31.
181 D. Schlichter and H. W. Fricke, *Naturwissenschaften*, 1990, **77**, 447.
182 P. Kaiser, D. Schlichter and H. W. Fricke, *Mar. Biol.*, 1993, **117**, 45.
183 C. L. Thurman, 'Adaptive coloration in invertebrates', *Proc. Symp. Am. Soc. Zool.*, ed. M. K. Wicksten, Galveston, TX, USA, 1990, p. 109.
184 D. L. Zmarzly, in 'Adaptive coloration in invertebrates', *Proc. Symp. Am. Soc. Zool.*, ed. M. K. Wicksten, Galveston, TX, USA, 1990, pp. 69–76.
185 J. M. Cervino, R. L. Hayes, M. Honovich, T. J. Goreau, S. Jones and P. J. Rubec, *Mar. Pollut. Bull.*, 2003, **46**(5), 573.
186 R. A. Cloney and E. Florey, *Z. Zellforsch.*, 1968, **89**, 250.
187 J. A. Mather and D. L. Mather, *J. Zool.*, 2004, **263**, 1, 89.
188 C. R. Darwin, *The Origin of Species*, Modern Library, 1859.
189 C. Haerlin and M. Haerlin, *Evol. Ecol.*, 2003, **17**(1), 197.
190 C. S. Loban and P. J. Harrison, *Seaweed Ecology and Physiology*, Cambridge University Press, Cambridge, 1994.
191 T. Guilford and J. Cuthill, *Evolution*, 1991, **45**, 449.
192 B. Chanas and J. R. Pawlik, *Mar. Ecol.: Prog. Ser.*, 1995, **127**, 195.

193 G. Gamberale and S. Tullberg, *Proc. R. Soc. London*, 1998, **265**, 889.

194 J. A. Endler and J. Mappes, *Am. Nat.*, 2004, **163**(4), 532.

195 G. Cimino, A. Spinella and G. Sodano, *Tetrahedron Lett.*, 1989, **30**, 3589.

196 P. Proksch, *Toxicon*, 1994, **32**, 639.

197 G. J. Bakus, *Science*, 1981, **211**, 497.

198 G. J. Bakus, *Mar. Biol.*, 1968, **2**, 23.

199 M. S. Grober, in 'Adaptive coloration in invertebrates', ed. M. K. Wicksten, *Proc. Symp. Am. Soc. Zool.*, ed. M. K. Wicksten, Galveston, TX, USA, 1990, p. 77.

200 T. Guilford and I. Cuthill, *Evolution*, 1991, **45**, 449.

201 G. Rosenberg, *Evolution*, 1991, **45**, 451.

202 T. M. Gosliner and D. W. Behrens, in 'Adaptive coloration in invertebrates', ed. M. K. Wicksten, *Proc. Symp. Am. Soc. Zool.*, ed. M. K. Wicksten, Galveston, TX, USA, 1990, pp. 127–145.

203 D. J. Faulkner and M. T. Ghiselin, *Mar. Ecol.: Prog. Ser.*, 1983, **13**, 295.

204 D. Mebs, *J. Chem. Ecol.*, 1985, **11**, 713.

205 D. P. Pisut and J. R. Pawlik, *J. Exp. Mar. Biol. Ecol.*, 2002, **270**, 203.

206 J. B. McClintock, D. Swenson, H. Trapido-Rosenthal and L. Banghart, *J. Chem. Ecol.*, 1997, **23**, 1607.

207 C. M. Young and B. L. Bingham, *Mar. Biol.*, 1987, **96**, 539.

208 J. B. McClintock and J. Vernon, *Mar. Biol.*, 1990, **105**, 491.

209 R. Hanlon, *Natl. Geogr.*, 2004, **206**(2), 30.

210 A. Ekendahl, *J. Exp. Mar. Biol. Ecol.*, 1998, **222**, 239.

211 R. Bedini, M. G. Canali and A. Bedini, A, *Cah. Biol. Mar.*, 2003, **4**, 375.

212 S. Merilaita, *Biol. Sci.*, 1998, **265**(1401), 1059.

213 G. D. Ruxton, M. P. Speed and D. J. Kelly, *Anim. Behav.*, 2004, **68**(3), 445.

214 W. M. Bandaranayake and P. Gentien, *Comp. Biochem. Physiol., B*, 1982, **72**, 409.

215 S. Johnsen, *Biol. Bull.*, 2001, **201**, 301.

216 M. J. McFall-Ngai, *Am. Zool.*, 1990, **30**, 175.

217 B. W. Jones and M. K. Nishiguchi, *Mar. Biol.*, 2004, **144**, 1151.

218 A. Barbosa, C. F. Florio, C. Chiao and R. T. Hanlon, *Bull. Mar. Biol.*, 2004, **207**, 154.

219 C. Chiao, E. J. Kelman and R. T. Hanlon, *Bull. Mar. Biol.*, 2004, **207**, 153.

220 C. Chiao, E. J. Kelman and R. T. Hanlon, *Bull. Mar. Biol.*, 2005, **208**, 7.

221 L. Litman and R. T. Hanlon, *Bull. Mar. Biol.*, 2004, **207**, 154.

222 M. Norman, *Nature Aust.*, 2002, **27**, 38.

223 P. H. Kelly and C. T. Swan, *J. Paleontol.*, 1988, **61**, 83.

224 M. Cusack, G. B. Curry, H. Clegg, G. Abbott and H. Armstrong, *Philos. Trans. R. Soc. London, Ser. B*, 1991, **333**, 359.

225 M. Cusack, G. B. Curry, H. Clegg, G. Abbott and H. Armstrong, *Comp. Biochem. Physiol., B*, 1992, **102**, 93.

226 M. James, A. D. Ansell and G. B. Curry, *Mar. Biol.*, 1991, **111**, 411.

227 C. W. Thayer and R. A. Allmon, in *Brachiopods Through Time*, ed. D. I. Mackinnon, D. E. Lee and J. D. Campbell, Balkema, Rotterdam, 1991, p. 253.

228 S. C. McKiliup and R. V. McKiliup, *Biol. J. Linn. Soc.*, 2002, **77**, 367.

229 O. G. Davidson, *Enchanted Braid: Coming to terms with Nature on the Coral Reefs*, John Wiley and Sons, New York, 1998, p. 85.

230 D. Owen, *Survival of the wild: Camouflage and mimicry*, University of Chicago Press, Chicago, 1980, pp. 146–149.

231 M. Ferrari, *Colors for survival: Mimicry*, Thomasson-Grant Publishing, Charlottesville, VA, 1992, p. 144.

232 A. S. Bull, *Creatures, corals and colors in American seas*, Audubon Park Press, New Orleans, LA, 1990.

233 M. Joron and J. Mallet, *Trends Ecol. Evol.*, 1998, **13**, 461.

234 J. Mallet and M. Joron, *Annu. Rev. Ecol. Syst.*, 1999, **30**, 201.

235 T. E. Reimchen, *Biol. J. Linn. Soc.*, 1989, **36**, 97.

236 D. J. Faulkner, *Nat. Prod. Rep.*, 1983, **9**, 323 (and references cited therein).

237 J. R. Pawlik, *Chem. Rev.*, 1993, **93**, 1911.

238 D. J. Faulkner, in *Ecological Roles of Marine Natural Products*, ed. V. J. Paul, Ithaca, Comstock, 1992, p. 119.

239 C. D. Amsler, K. B. Iken, J. B. McClintock and B. J. Baker, in *Marine Chemical Ecology*, ed. J. B. McClintock and B. J. Baker, CRC Press, New York, 2001, p. 195.

240 J. J. Stachowicz, in *Marine Chemical Ecology*, ed. J. B. McClintock, B. J. Baker, CRC Press, Boca Raton/London/New York, 2001, p. 157.

241 J. McClintock, *Those Amazing Sponges* (online), retrieved 1st September 2005 from www.antarctica.uab.edu/Templates/Journal.aspx/pid=39, The University of Alabama in Antarctica, 2004.

242 J. B. McClintock, C. D. Amsler, B. J. Baker and R. W. M. van Soest, *Integr. Comp. Biol.*, 2005, **45**, 359.

243 C. D. Amsler, J. B. McClintock and B. J. Baker, *Am. Zool.*, 2001, **41**, 17 (and references cited therein).

244 F. B. Furrow, C. D. Amsler, C. D. McClintock and B. J. Baker, *Mar. Biol.*, 2003, **143**, 443.

245 N. B. Perry, L. Ettouati, M. Litaudon, J. W. Blunt, M. G. H. Munro, S. Parkin and H. Hope, *Tetrahedron*, 1994, **50**, 3987.

246 A. Yang, B. J. Baker, J. E. Grimwade, A. C. Leonard and J. B. McClintock, *J. Nat. Prod.*, 1995, **58**, 1596.

247 A. D. Davis and A. L. Butler, *J. Exp. Mar. Biol. Ecol.*, 1989, **127**, 189.

248 G. Cimino, A. Spinella and G. Sodano, *Tetrahedron Lett.*, 1989, **30**, 3589.

249 P. F. Sale, *Oceanogr. Mar. Biol.*, 1980, **18**, 367.

250 P. Karuso, in *Bioorganic and Marine Chemistry*, ed. P. J. Scheuer, Springer-Verlag, Berlin, 1985, p. 31.

251 M. Ochi, K. Kataoka, A. Tatsukawa, H. Kotsuki and K. Shibata, *Chem. Lett.*, 1993, 2003.

252 V. M. Dembitsky, T. A. Gloriozova and V. V. Poroikov, *Mini-Rev. Med. Chem.*, 2005, **5**, 319.

253 K. R. Gustafson, N. Oku and D. J. Milanowski, *Curr. Med. Chem.*, 2004, **3**, 233.

254 D. M. Roll, C. M. Ireland, H. S. M. Lu and G. B. Elyakov, *J. Org. Chem.*, 1988, **53**, 3276.

255 P. A. Horton, R. E. Longley, O. J. McConnell and L. M. Ballas, *Experientia*, 1994, **50**, 843.

256 H. Kang and W. Fenical, *Tetrahedron Lett.*, 1996, **37**, 2369.

257 S. A. Abas, M. B. Hossain, D. Van der Helm, F. J. Schmitz, M. Laney, R. Cabuslay, R. and R. C. Schatzman, *J. Org. Chem.*, 1996, **61**, 2709.

258 Z. Ding, K. Chichak and J. W. Lown, *Curr. Med. Chem.*, 1999, **6**, 1.

259 P. Ivanova, L. S. Shevchenko, T. Sawabe, A. M. Lysenko, V. I. Svetashev, N. M. Gorshkova, M. Satomi, R. Christen and V. V. Mikhailov, *Int. J. Syst. Evol. Microbiol.*, 2002, **52**, 263.

260 A. Loukaci and M. Guyot, *Tetrahedron Lett.*, 1994, **35**, 6869.

261 U. Pindur and T. Lamster, *Curr. Med. Chem.*, 2001, **8**, 1681.

262 R. G. S. Bernlinck, R. Britton, E. Piers, L. Lim, M. Roberge, R. M. da Rocha and R. J. Anderson, *J. Org. Chem.*, 1998, **63**, 9850.

263 H. C. Vervoort, S. E. Richard-Gross, W. Fenical, A. Y. Lee and J. Clardy, *J. Org. Chem.*, 1997, **62**, 1486.

264 C. Eder, P. Schupp, P. Proksch, V. Wray, K. Steube, C. E. Muller, W. Frobenius, M. Herderich and R. W. M. van Soest, *J. Nat. Prod.*, 1998, **61**, 301.

265 R. A. Davis, A. R. Carroll and R. J. Quinn, *J. Nat. Prod.*, 1998, **61**, 959.

266 F. S. De Guzman, B. Carte, N. Troupe, D. J. Faulkner, M. K. Harper, G. P. Concepcion, G. C. Mangalindan, S. S. Matsumoto, L. R. Barrows and C. M. Ireland, *J. Org. Chem.*, 1999, **64**, 1400.

267 N. U. Sata, S. Wada, S. Matsunaga, S. Watabe, R. W. M. van Soes and N. Fusetani, *J. Nat. Prod.*, 1999, **64**, 2331.

268 J. Kobayashi, H. Nakamura, Y. Ohizumi and Y. Hirata, *Tetrahedron Lett.*, 1986, **27**, 1191.

269 J. Kobayashi, J. Cheng, Y. Kikuchi, M. Ishibashi, S. Yamamura, Y. Ohizumi, T. Ohta and S. Nozoe, *Tetrahedron Lett.*, 1990, **31**, 4617.

270 H. Nakamura, Y. Ohizumi, J. Kobayashi and Y. Hirata, *Tetrahedron Lett.*, 1984, **25**, 2475.

271 T. F. Molinski and D. J. Faulkner, *J. Org. Chem.*, 1987, **52**, 296.

272 J. B. McClintock and J. J. Gauthier, *Antarctic Sci.*, 1992, 179.

273 Y. Kashmen, S. Carmely, D. Blasberger, S. Hirsh and D. Green, *Pure Appl. Chem.*, 1989, **61**, 517.

274 T. Higa, J. Tanaka, A. Kitamura, T. Koyama, M. Takahashi and T. Uchida, *Pure Appl. Chem.*, 1994, **66**, 2227.

275 J. P. Michael and G. Pattenden, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 1 (and references cited therein).

276 J. Hawkins, *Pure Appl. Chem.*, 1988, **60**, 1267 (and references cited therein).

277 A. Bewley and D. J. Faulkner, *Angew. Chem.*, 1998, **110**, 2280.

278 G. R. Pettit, D. Kantoci, D. L. Doubek, B. E. Tucker, W. E. Pettit and R. M. Schroll, *J. Nat. Prod.*, 1993, **56**, 1981.

279 R. C. Bruening, E. M. Oltz, J. Furukawa, K. Nakanishi and K. Kustin, *J. Nat. Prod.*, 1986, **49**, 193.

280 D. E. Ryan, N. D. Ghatlia, A. E. McDermott, N. J. Turro and K. Nakanishi, *J. Am. Chem. Soc.*, 1992, **114**, 9659.

281 D. E. Ryan, K. B. Grant and K. Nakanishi, *Biochemistry*, 1996, **35**, 8640.

282 Q. Ding, K. Chichak and J. W. Lown, *Curr. Med. Chem.*, 1999, **6**, 1.

283 E. C. Glazer and Y. Tor, *Angew. Chem.*, 2002, **114**, 4194.

284 S. D. Bergman, D. Gut, M. Kol, C. Sabatini, A. Barbieri and F. Barigelletti, *Inorg. Chem.*, 2005, **44**, 7943.

285 S. P. Gunasekera, M. Gunasekara and P. McCarthy, *J. Org. Chem.*, 1991, **56**, 4830.

286 P. V. Bernhardt, P. Comba, D. P. Fairlie, L. R. Gahan, G. R. Hanson and L. Lotzbeyer, *Chem. Eur. J.*, 2002, **8**, 1527.

287 I. Mancini, G. Guella, C. Debitus and F. Pietra, *Helv. Chim. Acta*, 1995, **78**, 1178.

288 L. Colin and C. Arneson, *Tropical Pacific Invertebrates*, Coral Reef Press, California, USA, 1995.

289 M. Norman, *Cephalopods: A world guide*, Conchbooks, Hackenheim, Germany, 2000.

290 J. N. A. Hooper and F. Wiedenmayer, 'Porifera', in *Zoological catalogue of Australia*, ed. A. Wells, CSIRO, Melbourne, Australia, 1994, vol. 12.

291 *Systema Porifera*, ed. J. N. A. Hooper and R. W. M. Van Soest, Kluwer Academic/Plenum Publishers, New York, 2002, vols. 1 and 2.

292 R. A. Horne, *Marine Chemistry: The structure of water and the chemistry of the hydrosphere*, Wiley-Interscience, New York, 1969.

293 N. Lindquist, *J. Chem. Ecol.*, 2002, **28**, 1987.

294 H. J. Kim, *Ph.D. dissertation*, 2005, Department of Chemistry, North Carolina State University, Raleigh, USA.

295 J. Wallace, *Coral reefs: Exploring the world below*, Friedman/Fairfax, New York, 1995.

296 E. M. Antunes, B. R. Copp, M. T. Davies-Coleman and T. Samaai, *Nat. Prod. Rep.*, 2005, **22**, 62.

297 R. A. Manderville, *Curr. Med. Chem.: Anti-Cancer Agents*, 2001, **1**, 195.

298 S. Hibino and T. Choshi, *Nat. Prod. Rep.*, 2002, **19**, 148.

299 N. L. Thakur and W. E. G. Muller, *Curr. Sci.*, 2004, **86**, 1506.

300 *Bioactive Compounds from Natural Sources*, ed. C. Tringali, Taylor and Francis, New York, 2001.

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