Marine Bioprospecting − Trawling for Treasure and Pleasure

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This Microreview seeks to highlight the molecular diversity present in marine organisms, and illustrate by example some of the challenges encountered in exploring this resource. Marine natural products exhibit an impressive array of structural motifs, many of which are derived from biosynthetic pathways that are uniquely marine. Most importantly, some marine metabolites possess noteworthy biological activities, activities that have potential application outside marine ecosystems, such as antibiotics, antiparasitics, anticancer agents etc... The isolation, spectroscopic characterisation and assignment of stereostructures to these unusual metabolites is both challenging and rewarding. Examples featured in this Microreview follow a common theme in that they are all recent accounts of the isolation of natural products from Australian marine sponges, carried out in the laboratories of the author. In addition to presenting brief comments on specific structure elucidation strategies, an effort is made to emphasize techniques for solving stereochemical issues, as well as to speculate on the biosynthetic origins of some of these exotic marine natural products.

Introduction

Terrestrial biodiversity has long been recognised and exploited as a source of valuable bioactive substances — from herbal remedies, natural medicines and traditional drugs through to modern pharmaceuticals and agrochemicals. Surrounded as they were by little more than indigenous plant and animal life, from the deserts to the tropics early cultures survived and prospered by carefully exploring and exploiting their environment. Initially focused on the need for food and shelter, over time, and through trial and error, most cultures acquired knowledge of the *curative* powers of selected plants. Access to these curative powers typically demanded elaborate preparations of *special* plants, accompanied by complex rituals that assumed great cultural significance. It is little wonder that these rituals were attributed curative powers, given the almost supernatural impact that these crudest and earliest of plant extract medicines must have had on primitive societies. Indeed, from what we now know as the *placebo effect,* it is quite possible that for committed believers the ritual was indeed important. Cultural traditions aside, it is clear that the overwhelming curative effect of natural medicines was due to biologically active metabolites present within the plant material. As scientific methods matured it became possible to detect, quantify, and fractionate out active agents, and identify their molecular structures. Armed with this knowledge, chemists were able to manufacture some, but not all, of nature's gifts. This was a critical contribution since many source plants were only found in limited regions of the world, restricting supply and pushing the cost out of the reach of all but the very rich. Likewise, the effectiveness of these natural medicines

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Robert J. Capon was born in England in 1956 and emigrated to Australia in 1959. After graduating with BSc Honors (1st Class) from the University of Western Australia in 1979, he went on to complete a PhD at the same institution in 1982. His PhD thesis was the first to explore the natural products chemistry of Western Australian marine sponges and algae. Pursuing this theme further, in 1982 he accepted a Postdoctoral Fellowship at Scripps Institution of Oceanography, California, with Professor John Faulkner, and in 1984 returned to Australia to take up a Research Fellowship at the Australian National University with Professor John MacLeod. While at the Australian National University he initiated a program of natural products research aimed at novel bioactive metabolites from southern Australian marine sponges. In 1986, at the invitation of the Australian Antarctic Division he travelled to Australian Antarctic Territories, fuelling an ongoing interest in Antarctic marine chemistry. In 1988 he accepted an appointment as Lecturer in chemistry at The University of Melbourne, rising through Senior Lecturer (1992) to his current level of Associate Professor (1996). His academic career has been dominated by a commitment to marine bioprospecting that has seen him secure substantial support from government and industry, as well as publish and lecture widely on this topic. He leads a productive research team whose efforts have revealed a significant portion of what is known about the molecular diversity found in southern Australian marine organisms. In 1998 Associate Professor Capon was awarded a prestigious Universitas 21 Fellowship in recognition of his dual roles as a distinguished scientist, and champion of interactive multimedia technologies in education and training. Associate Professor Capon has authored over 120 scientific publications, two book chapters, and three CD ROM's.

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varied enormously with the quality (or lack thereof) of the raw ingredients. Bioprospecting, the search for biologically active substances from nature, became a respectable profession, that was both commercially and scientifically profitable, and of value to the community in general. By the 20th century bioprospectors had turned their attention to the newly discovered world of microbes, where a vast collection of bioactive metabolites served as a foundation for the modern pharmaceutical industry. Rather than rely solely on the laborious harvesting and processing of plants, or on synthetic chemists to manufacture drugs in laboratories and factories, microbiologists were called in to supervise massive scale fermentation of selected microbes. With microbes drafted in to the production process, the supply of valuable drugs improved and costs plummeted, resulting in the widespread use of microbial metabolites in health care and agriculture. Early successes included the antibacterial penicillins, the antifungal nystatins, and the antiparasitic avermectins. In addition to pharmaceuticals that saved and/or improved the quality of life for countless millions, natural product derived agrochemicals increased productivity the world over — improving animal husbandry and protecting crops from disease and pests. Of course, along the way multinational chemical companies earned billions.

Despite many impressive successes the quest for new drugs continues, as diseases and pests relentlessly evolve and share resistance to existing pharmaceuticals and agrochemicals. While the traditional sources of terrestrial plants and microbes will undoubtedly continue to yield valuable new bioactive agents, it is ever more important to explore all available pools of molecular diversity. This strategy to seek out new ecosystems for bioprospecting brings with it the opportunity to discover unprecedented molecular structures, with bioactivities unencumbered by known (and evolving) mechanisms of drug resistance.

With oceans covering a sizeable percentage of the earth's surface it was only a matter of time before bioprospectors recognised the remarkable potential of marine biodiversity. With ecosystems spanning cold polar waters through temperate latitudes to the tropics, marine biodiversity is without doubt far more extensive than popularly perceived. While some early coastal or islander societies did develop traditional medicines from marine organisms, such occurrences were rare and limited to intertidal and/or shallow water organisms. Even today, at the start of a new millennium, only a select group of intrepid adventurers have observed at first hand the unique array of organisms that comprise benthic communities beyond shallow coastal waters (<50m). Pioneering work by a handful of academic chemists in the 1970′s initiated a field of study that saw marine natural products chemistry emerge from curiosity status to a major scientific force by the turn of the century. Since those early days, marine metabolites have captured the imagination of synthetic chemists, biochemists, pharmacologists and others alike, with interdisciplinary research becoming common place. In this respect the term bioprospector becomes apt, since the field is no longer the exclusive domain of natural product chemists, but has broadened to encompass ecologists, biologists and physical scientists. Far from completing the task, the past 30 years of accelerated interest in marine natural products chemistry has served to reveal base line knowledge. This knowledge provides a foundation on which many talented young scientists will undoubtedly build future careers. With improvements in technology (NMR, HPLC, LC/MS...) today's bioprospectors are able to detect, isolate and identify milligram or even sub-milligram amounts of target metabolites. This leaves open the opportunity to return to organisms that have already been studied, or to sample organisms previously deemed physically too small or rare for natural products research. Likewise, just as terrestrial bioprospecting moved from plants to microbes, so too marine microbial natural products chemistry is assuming prominence. With the development of sophisticated bioassays (drawing on exciting advances in genomics, biochemistry and pharmacology) we can detect very potent metabolites in crude marine extracts, targeting compounds that eluded earlier researchers. Bioassay-directed isolation strategies targeting commercially important properties have largely overtaken chemical intuition driven enquiry (based on NMR and/or TLC analysis of crude extracts), leading to productive collaborations between academic- and industry-based researchers.

Although the term marine bioprospecting conjures up images of wholesale harvesting of marine organisms, akin to mineral prospecting or commercial fishing activities, this is inaccurate. Unlike mining and fishing, which rely entirely on the physical extraction of natural resources, whether ore bodies or fish stocks, marine bioprospecting is better characterised as a quest for knowledge. True, the research requires access to collections of marine organisms (sponges, algae, tunicates etc...), but these are typically limited to the collection of single specimens (or at most a handful of specimens) of any given species. Detailed screening and analysis of these sample collections leads to the isolation and identification of active agents, and it is this *knowledge* that initiates drug discovery programs. These programs aim to define, refine and optimise the pharmacophore through the production and screening of libraries of synthetic analogues. Few commentators would entertain the proposition that wild harvesting of marine metabolites could realistically support a commercial product. In certain situations, mariculture may be attractive, or in a more speculative moment genetic transfer to microbes may hold the answer. A more probable renewable source of valuable, and yet structurally complex, marine metabolites is the fermentation of marine microbes. Having generated a viable laboratory culture of a marine microbe, and having demonstrated that it produces a valuable bioactive agent, the option exists to supply demand by industrial-scale fermentation. An exciting prospect, but still one at the most formative phase. The potential for future development is enormous! Whatever the strategy for commercialising a marine metabolite (or analogue) the reality must be that *the more we learn about and demonstrate the value of marine biodiversity, the stronger is the case for intelligently conserving all biodiversity, both terrestrial and marine*. It will take centuries to fully explore all

that exists today, assuming of course that it exists tomorrow.

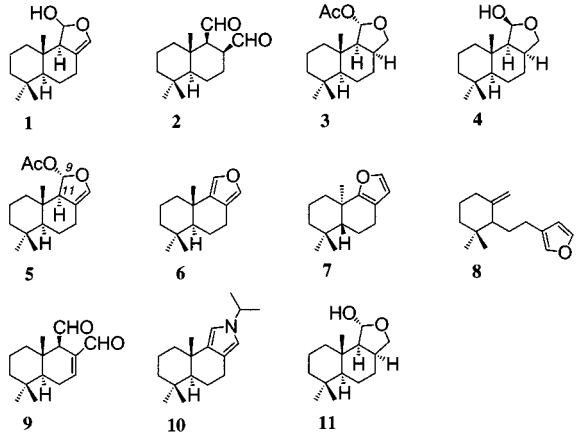
The remainder of this Microreview seeks to illustrate some of what we have learned about marine chemistry, drawing on selected recent accounts of research into the chemistry of Australian marine sponges carried out in the laboratories of the author. These examples have been selected so as to demonstrate the breadth of biosynthetic versatility encountered in even a single marine phyla — sponges. Marine metabolites are presented in biosynthetic groupings, although it should be noted that these are intuitive associations drawn from common structural features rather than based on experimental studies.

Terpenes

Sponges are rich in sesquiterpenes, diterpenes, sesterterpenes and steroids. Many marine terpenoids feature uncommon functionality and/or carbon skeletons that are uniquely marine, and as such their detection, isolation and structure elucidation present exciting and rewarding challenges. Consider the following examples.

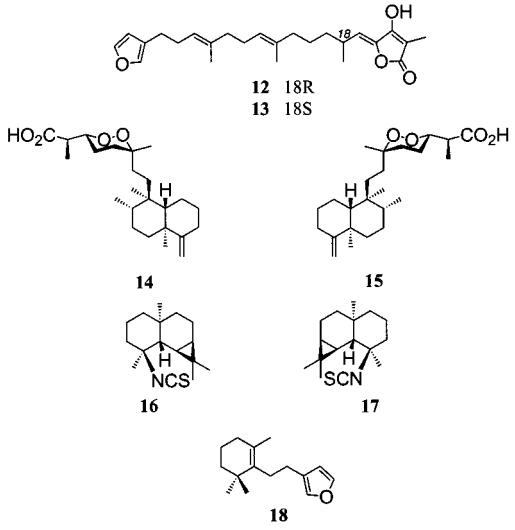
Sesquiterpenes

A *Dysidea* sp collected during scientific trawling operations north of Flinders Island in Bass Strait returned a selection of new (**1**−**4**) and known [(−)-7-deacetoxyolepupane (**5**), (+)-euryfuran (**6**), (−)-pallescensin-A (**7**), and dihydropallescensin-2 (**8**)] oxygenated drimanes.[1] Isolation and identification of the acetal **1** was especially difficult since it proved to be very unstable and underwent rapid conversion into the dialdehyde **2** during characterization. The dialdehyde **2** bears a striking resemblance to the known terrestrial (and marine) antifeedant polygodial (**9**).[2] Polygodial (**9**) is reported to be a hot-tasting defence allomone, known to occur in terrestrial plants and marine nudibranches, and very likely acts by double condensation with primary amines (e.g. lysine residues in peptides) to generate a pyrrole conjugate.[3] When exposed to isopropylamine the aldehyde **2** demonstrated the same capability, returning a quantitative yield of the pyrrole **10**. Given these considerations it was speculated that (−)-7-deacetoxyolepupane (**5**) was a masked precursor to the acetal **1**, which in turn was a transient pro-drug to the dialdehyde chemical defence agent **2**. The remaining co-metabolites **3**, **4** and **6**−**8** are clearly related by common biosynthetic origins. Spectroscopic analysis and chemical interconversion established for the first time the absolute stereochemistry for all these co-metabolites. Although the relative stereostructure for (−)-7-deacetoxyolepupane (**5**) had previously been reported,[4] assignment of the H-9/H-11 relative stereochemistry rested on NOE measurements between H-9 and H-11. Similar arguments had been used for a selection of other marine drimanes incorporating an acyl acetal subunit (olepupane,[5] 6β-acetoxyolepupane[6] and a number of acyl ester analogues[5]). At first glance it seemed that a similar approach could be used to identify the single natural epimer of the free acetal **4**, since it might be predicted that the natural acetal epimer **4** would possess the same stereochemistry as the related acyl acetals mentioned above. Rather than rely on assumptions, efforts were made to determine this stereochemical assignment experimentally. On brief storage in aprotic solvents (hexane or CH_2Cl_2) the acetal **4** underwent partial epimerization to the C-11 epimer **11**. These epimers could be resolved by HPLC and when stored in protic solvents (MeOH) did not equilibrate. Unexpectedly, both **4** and **11** displayed comparable NOE difference measurements to H-9 on irradiation of H-11, invalidating NOE arguments as a method for determination of relative H-9/H-11 stereochemistry in the free acetal. This observation also raised doubts about the ability of NOE measurements to unambiguously assign stereochemistry to acyl acetals such as **5**. Assignment of the H-9/H-11 relative stereochemistry in **4** was eventually secured by the use of lanthanide shifts reagents, which revealed the more stable acetal epimer to have the opposite C-11 stereochemistry to that previously assigned to the acyl acetal. Although **4** was acetylated to yield **3**, the reaction conditions facilitated equilibrium between **4** and **11** such that the relative stereochemistry assigned to **4** cannot be unambiguously extended to **3**. At this time no unambiguous assignment of the H-9/H-11 relative stereochemistry has been reported for the acyl acetals described above.



The experimental assignment of stereochemistry to novel marine metabolites is perhaps as challenging, if not more challenging, than the isolation and determination of gross structures. Given limited access to material, stability concerns, and structural complexity, it is not uncommon for marine metabolites to be reported without consideration of absolute or even relative stereochemistry. *Biosynthetic arguments* are frequently invoked (either overtly or covertly) to infer preferred stereochemistry by comparison with *biosynthetically related* marine metabolites of known absolute stereochemistry. Although not unreasonable in certain circumstances, care must be taken when employing this strategy. For example, correlation with known compounds that are *not* co-metabolites assumes that both source organisms yield a common antipodal series. The marine natural products literature reveals several instances where enantiomers

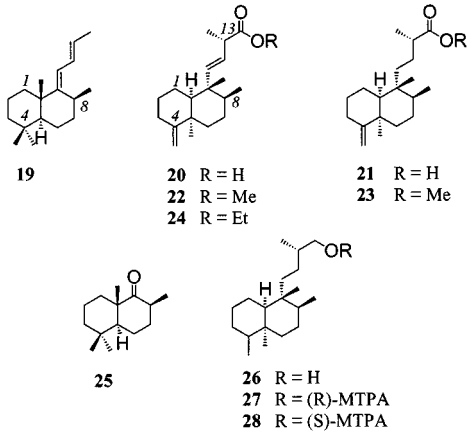
are produced by different source organisms. These source organisms can be different samples of the same genus [e.g. (−)-variabilin (**12**)[7] versus (+)-variabilin (**13**)[8] from sponges of the genus *Ircinia*], or different genera [e.g. the sponge metabolites sigmosceptrellin A (**14**) from a *Sigmosceptrella* sp,[9] versus enantio-sigmosceptrellin A (**15**) from a *Mycale* sp[10]], or from entirely different phyla [e.g. (+)-epipolasin A (**16**) from the sponge *Epipolasis kushimotoensis*[11] versus (−)-epipolasin A (**17**) from both the sponge *Acanthella pulcherrima*[12] and the nudibranch *Cadlina luteomarginata*[13]].



The *biosynthetic argument* is therefore best applied only on those occasions when the biosynthetically related compound of known absolute stereochemistry is a co-metabolite. Failure to assign absolute stereochemistry, along with erroneous literature assignments that might or might not be reassigned, demand that great care be taken to ascertain that absolute stereochemistry assigned to key model compounds is based on sound reasoning. As a cautionary note, compounds that appear with *assigned* absolute stereochemistry in review articles can prove less absolute if one takes the effort to read the primary literature. Structure diagrams with arbitrary absolute stereochemistry in the primary literature have on more than one occasion found their way into reviews without due comment on their arbitrary character. Having noted all of the above, the seemingly anomalous co-occurrence of the antipodal drimanes (+)-euryfuran (**6**) and (−)-pallescensin-A (**7**) deserves comment. A *biosynthetic argument* might have assigned these two compounds to a common antipodal series, and yet experimental evidence confirmed the assignments as shown. A plausible explanation is that both **6** and **7** are derived from alternate cyclizations of a common achiral precursor [e.g. **18**, c.f. dihydropallescensin-2 (**8**)], with different orientations in the active enzyme site accounting for regio- and stereochemical variation. This example highlights the limitation that *biosynthetic arguments* are by definition *only* as accurate as the biosynthetic pathways that underpin them. Since little experimental data exist for the vast majority of marine biosynthetic pathways, most biosynthetic relationships draw on comparisons with the terrestrial world (i.e. terpene biosynthesis), or plausible speculations. This analysis of the limitations inherent to *biosynthetic arguments* should not be interpreted as invalidating the approach, but rather encourage caution in those who invoke it, and those who draw on such reports.

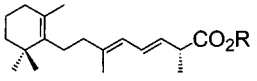
Diterpenes

A *Sigmosceptrella* sp collected during scientific trawling operations in the Great Australian Bight yielded a series of norterpenes identified as sigmosceptrins A−C (**19−21**).[14] Methylation of the crude sponge extract facilitated isolation of the carboxylic acids **20** and **21** as their methyl ester derivatives **22** and **23**. Prolonged storage in EtOH also resulted in formation of the artefact ethyl ester **24**. Assignment of the complete stereostructure for sigmosceptrin A (**19**) required consideration of ^{1}H NMR coupling constants, NOE difference measurements, and chemical degradation to the known ketone **25**. Interpretation of spectroscopic data together with X-ray crystallographic analysis identified the relative stereostructure for sigmosceptrin B methyl ester (**22**), and also by inference for the free acid **20** and ethyl ester **24**, and also the structurally related and spectroscopically very similar co-metabolite sigmosceptrin C (**21**) and its methyl ester **23**. Hydrogenation of **22** (Pd/C, H_2) followed by reduction ($LiAlH_4$) yielded the saturated alcohol **26**, which was in turn converted into (*R*)- and (*S*)-MTPA esters (**27** and **28**). Comparison of the H_2-14 ^{1}H NMR multiplets in **27** and **28** provided evidence for assignment of a 13*R* stereochemistry, and hence assignment of the complete absolute stereochemistry for sigmosceptrins B and C.

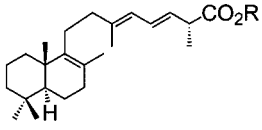


To understand the biosynthetic origins of the norterpene sigmosceptrins A−C (**19−21**) it is worthwhile considering the two norterpene dienes **29** and **30** discovered in a specimen of *Latrunculia brevis* collected by SCUBA off South Durras on the mid-south coast of New South Wales.[15] As with sigmosceptrins B and C, the norterpene dienes from *Latrunculia brevis* were isolated and characterised as their corresponding methyl ester derivatives **31** and **32**. Whereas methylation of the sigmosceptrins was simply an aid to isolation, the diene acids **29** and **30** proved highly unstable

and could *only* be preserved in pure form for any length of time as their methyl esters. While even short-term exposure of the diene acids (**29** and **30**) to aprotic NMR solvents ($CDCl_3$, C_6D_6) led to significant decomposition, they could be successfully stored for several weeks in the dark at less than −15 °C in protic solvents (MeOH or EtOH). Curiously, these same compounds survived years of storage in crude aqueous EtOH sponge extracts at less than −15 °C. It remains unclear whether this stability in crude form is a concentration issue, or the stabilising effect of unknown constituents in the sponge extract. Although equally unstable in aprotic solvents the methyl esters **31** and **32** survived several month's storage in protic solvents in the dark at less than −15 °C. As a corollary to this issue of stability, it was noted that ^1H and ^{13}C NMR resonances associated with the diene acid subunits of either **29** or **30** were excessively broadened when acquired in aprotic solvents ($CDCl_3$, C_6D_6), but were sharp and well defined in protic solvents (CD_3OD). This phenomenon was reproducible and reversible. In contrast, the methyl esters returned excellent NMR spectroscopic data irrespective of solvent. It has been speculated that the diene functionality in these metabolites is susceptible to interaction (protonation?) by the adjacent acid functionality, and that this interaction is attenuated by H-bonding between the acid moiety and protic solvents, or by derivatization of the acid moiety. If this were the case then other natural products with a similar constellation of diene and acid moieties should experience the same NMR broadening and stability problems. As will be discussed later in this review, just such a situation was observed with cometin A,[16] which possesses a diene in proximity to a tetronic rather than carboxylic acid! Assignment of complete stereostructures to **29** and **30** required detailed spectroscopic analysis of the methyl esters **31** and **32**, as well as chemical derivatization and Mosher analysis, not dissimilar to that applied to sigmosceptrins B and C (**20** and **21**).



29 R = H
31 R = Me



30 R = H
32 R = Me

A possible acyclic biosynthetic precursor to the norditerpene acid **29** can also be viewed as a plausible biosynthetic precursor to all the sigmosceptrins. A schematic representation of this proposed biosynthetic relationship is outlined in Figure 1. Whereas the acyclic precursor (i) can cyclize by a common terpenoid pathway to the monocyclic norditerpene diene acid **29**, a second cyclization event could lead to the "labdane" intermediate (ii). That such double cyclization events are feasible in this structure class is demonstrated by the norsesterterpene diene acid **30** which features a typical "labdane" bicyclic ring system. The intermediate (ii) can proceed via a typical Meerwin−Wagner cascade from a "labdane" to a "clerodane" ring system, leading to sigmosceptrins B and C (**20** and **21**). Alternatively, decarboxylation of (ii) can lead to sigmosceptrin A (**19**). These proposed biosynthetic relationships between norterpene dienes and sigmosceptrins assume added significance when considering the biosynthetic origins of marine norterpene cyclic peroxides. While norditerpene cyclic peroxides are known from marine sponges, this class of marine metabolite is dominated by norsesterterpenes, and as such discussion will be held over until later in this review.

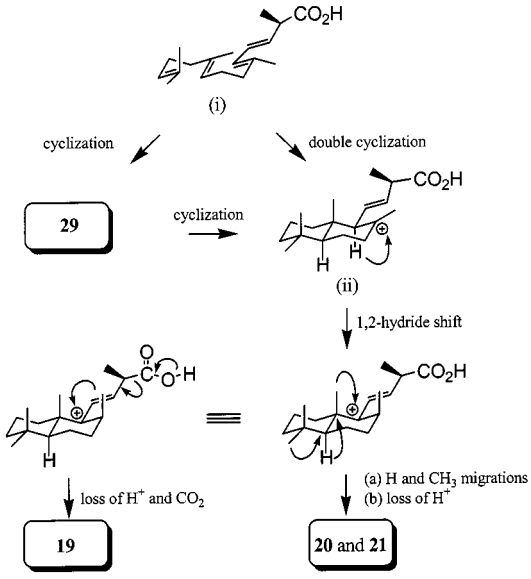
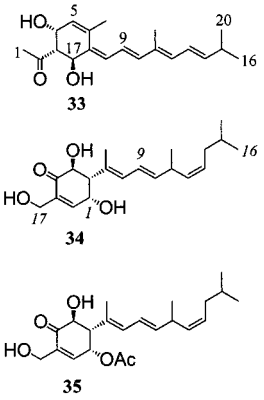


Figure 1. Proposed biosynthetic relationships between norterpene diene acid **29** and sigmosceptrins A−C (**19−21**)

Illustrative of the potential of marine sponges to continue yielding novel metabolites with unprecedented carbon skeletons is the recent discovery of the phorbasins A−C (**33−35**) from two specimens of *Phorbas* sp collected during scientific trawling operations in the Great Australian Bight. The first *Phorbas* specimen yielded an extremely unstable polyene diterpene, identified by spectroscopic analysis as the new diterpene phorbasin A (**33**).[17] Although the isolation and spectroscopic characterisation of **33** was carried out swiftly, without recourse to elevated temperatures (>40 °C), or exposure to bright sunlight or other than neutral pH, this compound underwent complete decomposition during handling. Analysis of spectroscopic data confirmed the relative stereostructure for phorbasin A (**33**) as shown. This compound incorporates an unprecedented diterpene carbon skeleton. A parallel investigation of another *Phorbas* sp collected during the same scientific cruise yielded two new monocyclic diterpenes identified as phorbasin B (**34**) and C (**35**).[18] Once again, complete relative stereostructures were secured by spectroscopic analysis. Stability issues precluded experimental determinations of absolute stereochemistry for phorbasins A−C.



Although phorbasins A−C (**33**−**35**) incorporate different carbon skeletons it is possible to propose a plausible biosynthetic relationship between these *Phorbas* metabolites. Phorbasin A (**33**) requires closure from C-6 to C-17 in an acyclic precursor with an associated C-7 to C-6 methyl migration, while phorbasins B−C (**34**−**35**) can be rationalised by closure from C-6 to C-1 (as indicated in Figure 2). These alternative cyclization mechanisms can be viewed as being related through rotation about the C-3/C-4 bond, with additional functionality being defined either pre or post cyclization.

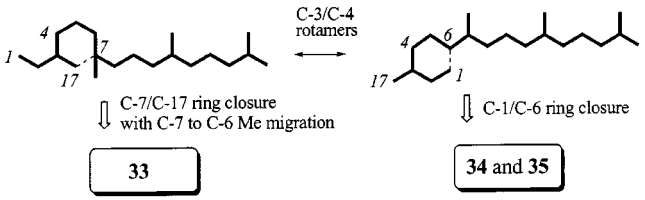
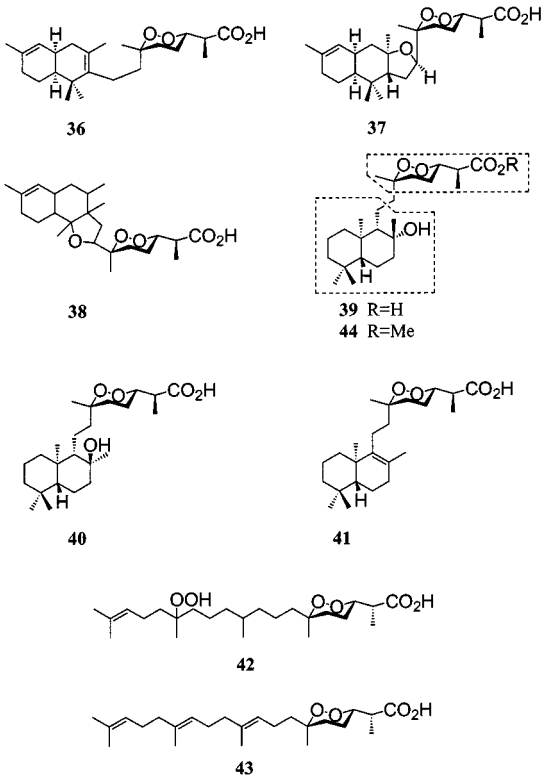


Figure 2. Possible biosynthetic relationship between phorbasins A−C (**33**−**35**)

Sesterterpenes

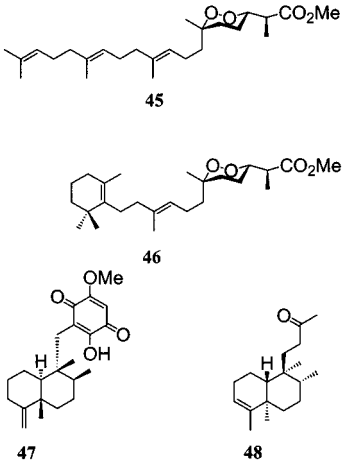
Marine sponges are particularly adept at producing oxygenated sesterterpenes. Well represented among marine sesterterpenes are a wide array of metabolites that feature such functional groups as cyclic peroxides, tetronic acids, furans and γ-lactones. What follows is a brief account of some of our adventures in exploring these structure classes.

Our interest in norsesterterpene cyclic peroxides was prompted by an early encounter (1985)[10] which resulted in the development of an empirical method for assigning relative stereochemistry about the three chiral centres featured in the cyclic peroxide carboxylic acid terminus (C-2, C-3 and C-6). This methodology has since been extensively employed to assign stereochemistry to all known examples of this structure class. Our next encounter yielded trunculins A (**36**) and B (**37**),[19] which were the first reported examples of the novel trunculin carbon skeleton. Although the number of known trunculins has since grown to nine, the latest being trunculin I (**38**),[20] this carbon skeleton is thus far exclusive to southern Australia *Latrunculia* spp. Over the last decade we have encountered norsesterterpene cyclic peroxides from three genera of southern Australian marine sponge, including *Latrunculia*, *Mycale* and *Sigmosceptrella*. In addition to the trunculins mentioned above, selected examples include mycaperoxides C (**39**), D (**40**) and G (**41**) from *Mycale* spp,[21,22] and sigmosceptrellins D (**42**) and E (**43**) from a *Sigmosceptrella* sp.[23]



Although methodology exists to determine relative stereochemistry about the cyclic peroxide terminus, assignment of the total relative stereostructures has often proved very challenging. Many norsesterterpene cyclic peroxides incorporate two chiral subunits that are effectively independent, lacking through-space interactions and being attached through a rotationally flexible linkage [c.f. mycaperoxides C (**39**)]. While spectroscopic methods typically permit ready assignment of relative stereochemistry to each separate chiral subunit, relating these subunits has proved problematic. In the absence of X-ray data (most norsesterterpene cyclic peroxides are oils) many assignments have relied on the calculation and interpretation of molar rotations ($[\varphi]_D$). This approach relies on the established empirical observation that the molar rotation of a molecule can be considered equivalent to the sum of the molar rotations of independent chiral subunits within the molecule (for all practical purposes this approach is limited to molecules comprising only two chiral subunits). Chiral subunits are deemed to be independent for the purpose of this analysis if they are separated by a minimum of an ethylene bridge, and if they do not engage in through-space interactions such as H-bonding or steric interference. The value of this approach lies in the fact that molecules of known absolute stereochemistry that encompass only a single chiral subunit make it possible to quantify the molar rotation for that chiral subunit. Consequently, if an examination of the optical data for model compounds permits the determination

of the molar rotations for both chiral subunits in a molecule of unknown absolute stereochemistry, the arithmetic sum of these contributions can be used to assign the absolute stereochemistry. Consider the case for mycaperoxide C methyl ester (**44**). The molar rotation for the cyclic peroxide subunit is calculated to be either −244° (2*S*,3*S*,6*S*) or +244° (2*R*,3*R*,6*R*) based on the model compounds **45** and **46**. The absolute stereochemistry of **45** and **46** had been determined independently by Horeau analysis.[10] Likewise, three compounds of known absolute stereochemistry exist in the literature for which the bicyclic chiral subunit in **44** is the sole chiral subunit. Published optical rotations for these models permit estimation of a molar rotation for the bicyclic subunit as +29° to +107° (9*R*,10*S*,13*S*,18*R*), or −29° to −107° (9*S*,10*R*,13*R*,18*S*). The experimentally measured molar rotation for **44** of −304° is indicative of a 2*S*,3*S*,6*S*,9*S*,10*R*,13*R*,18*S* absolute stereochemistry.[21] Clearly, the application of molar rotations is constrained by the availability of suitable model compounds of known absolute stereochemistry, and the accuracy with which optical properties have been measured and reported (taking care to note the need for common solvents and comparable concentrations). In the pursuit of absolute stereochemistry we have variously employed molar rotations ($[\varphi]_D$) as well as Horeau, Mosher and CD analyses, plus chemical degradation and asymmetric synthesis. Comparisons between these various techniques has proved to be very revealing, and have in turn led to a re-evaluation and reassignment of absolute stereochemistry for other classes of marine natural product [e.g. ilimaquinone (**47**) and related metabolites[24]].



Among recently reported norsesterterpene cyclic peroxides, the most noteworthy, without doubt, was sigmosceptrellin D (**42**).[23] Apart from incorporating a highly unusual hydroperoxide moiety (to the best of our knowledge the few naturally occurring known marine hydroperoxides are allylic), this natural product provided the first indication of an elegant and plausible biosynthetic pathway capable of accounting for all known norterpene cyclic peroxides, including C-2, C-3 and C-6 stereoisomers, the diene acids **29** and **30**, sigmosceptrins (**19**−**21**), and a host of degraded norterpene co-metabolites (i.e. **48**).[23] Key to proposing this biosynthetic pathway (see Figure 3) was the recognition that the hydroperoxide moiety in **42** was strategically positioned in such a way as to mimic the cyclic peroxide moiety at the opposite end of the molecule. This observation encouraged a view that the biosynthetic origin of marine norterpene cyclic peroxides involved a common C-6 hydroperoxide intermediate (Figure 3: STEP 1) undergoing a Michael addition to an adjacent α,β-unsaturated carboxylic acid or ester moiety (Figure 3: STEP 2). Initial formation of the C-6 hydroperoxide could occur on either the *Re* or *Si* face of a $\Delta^{5,6}$ precursor, thereby defining the absolute stereochemical outcome of the biosynthetic process. Figure 3 arbitrarily illustrates this process occurring by oxidation of the *Re* face of a $\Delta^{5,6}$ precursor, although the antipodal pathway could be evidenced by known norterpene cyclic peroxides. Depending on the conformation of the hydroperoxy carboxylic acid precursor, the electrophilic $\Delta^{2,3}$ addition could proceed at the *Si* face (Figure 3: STEP 2a) or the *Re* face (Figure 3: STEP 2b), leading to epimers about C-3. Quenching of the resulting $\Delta^{1,2}$ enolate could proceed by both *Re* and/or *Si* facial addition of H^+ to yield the full suite of C-2 and C-3 stereoisomers. Those isomers originating from the process STEP 2b→3b→4b also experience inversion of the cyclic peroxide chair conformation to attain the more stable conformer bearing an equatorial C-3 substituent. This inversion has the effect of repositioning the C-6 terpene side chain from an equatorial to an axial conformation. That the C-14 hydroperoxide in sigmosceptrellin D (**42**) did not yield a cyclic peroxide is readily explained in that, lacking

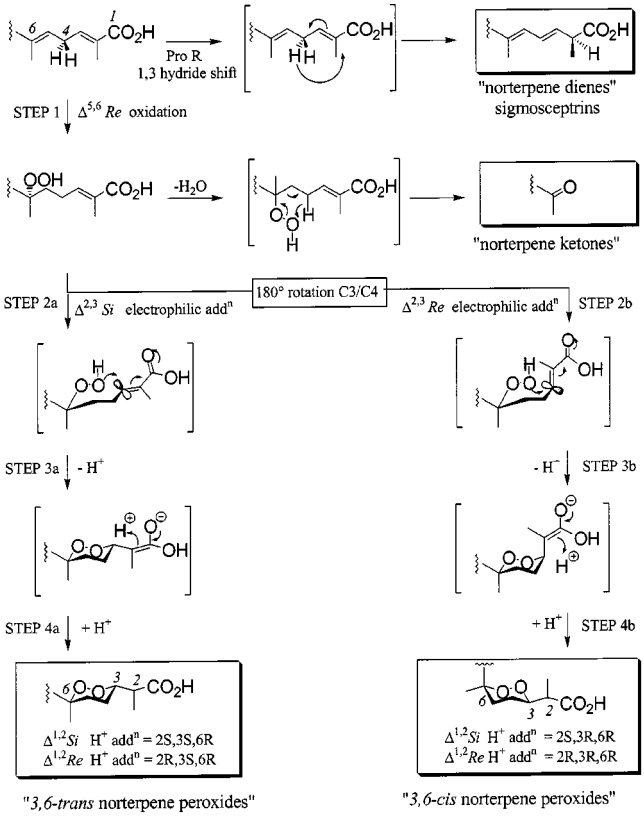
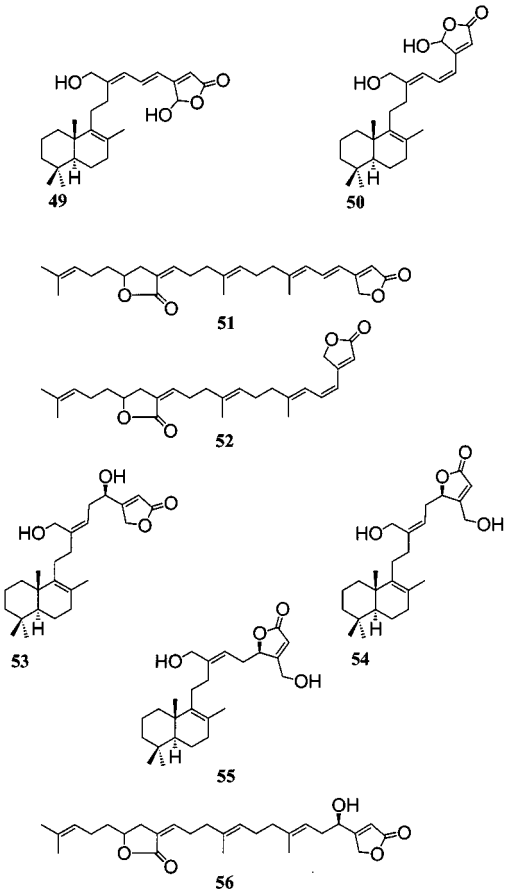


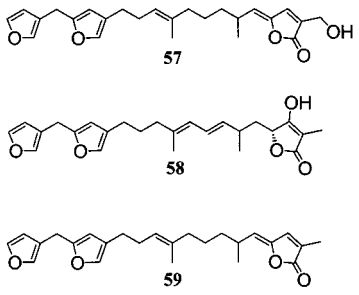
Figure 3. Plausible biosynthetic relationships between norterpene peroxides and related metabolites

a C-19 carboxylic acid, $\Delta^{17,18}$ was not sufficiently activated towards electrophilic attack by the C-14 hydroperoxide. This proposed biosynthetic pathway not only provides an efficient route to the key structural feature in norterpene cyclic peroxides, but also provides ready access to known co-metabolites. Oxidative degradation through loss of water from the hydroperoxide carboxylic acid precursor, with accompanying intramolecular abstraction of an allylic C-4 proton and cleavage of C-5/C-6 (see Figure 3), can yield norterpene ketones. Such ketones are increasingly being identified as minor co-metabolites with norterpene cyclic peroxides.[21,22] Likewise, a Pro *R* 1,3-hydride shift from C-4 to C-2 could yield norterpene dienes, which are known *Latrunculia* metabolites[15] and are speculative biosynthetic precursors for the closely related sigmosceptrins (see Figure 2).[14] It should be emphasised that in the proposed biosynthetic scheme the conjugated norterpene dienes described above are not biosynthetic precursors to the cyclic peroxides, but are rather an offshoot of the biosynthetic pathway. This proposal differs from an earlier hypothesis that required the [2 + 4] cycloaddition of oxygen to conjugated norterpene dienes. Although no experimental evidence is presented to support the biosynthetic pathway outlined in Figure 3, it is an attractive proposal in that it goes a long way towards explaining the stereochemical versatility encountered among known marine norterpene cyclic peroxides, and accounts for the occurrence of related metabolites.



A specimen of *Luffariella geometrica* collected during commercial trawling operations in the Great Australian Bight yielded an extraordinary array of new sesterterpene, norsesterterpene, diterpene and norditerpene butenolides, identified as luffarins A−Z.[25] These include stereoisomeric pairs such as luffarin E (**49**) and F (**50**), and luffarin T (**51**) and U (**52**), as well as regioisomeric butenolides such as luffarin I (**53**) and L (**54**). Originally isolated due to their antimicrobial properties, several luffarins were subsequently found to be potent inhibitors of the neuronal nicotinic receptor. Structure activity investigations carried out on the suite of available luffarins established the hydroxy butenolide subunits in luffarins K (**55**) and R (**56**) as key pharmacophores. On the basis of these investigations we successfully predicted similar bioactivity for another sponge metabolite, cometin B (**57**).

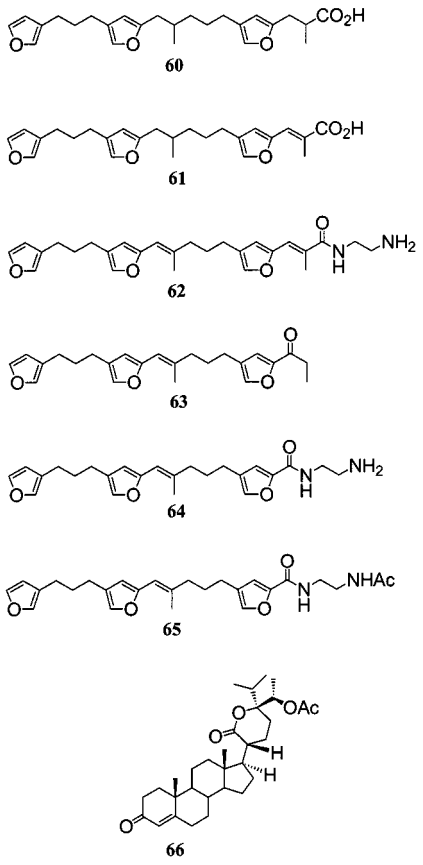
Cometins A (**58**), B (**57**) and C (**59**) were isolated from a *Spongia* sp collected during commercial trawling operations in the Great Australian Bight.[16] While cometin A (**58**) possessed a tetronic acid moiety typical of other examples of this structure class (c.f variabilin above), cometins B (**57**) and C (**59**) were less common butenolide analogues. Curiously, the juxtaposition of diene and tetronic acid moieties in cometin A (**58**) was reminiscent of the diene acids **29** and **30**.[15] In common with **29** and **30**, cometin A (**58**) displayed excessive broadening of ^{1}H and ^{13}C NMR resonances in aprotic solvents, which sharpened in protic solvents. Studies into the biological properties of luffarins and cometins are ongoing.



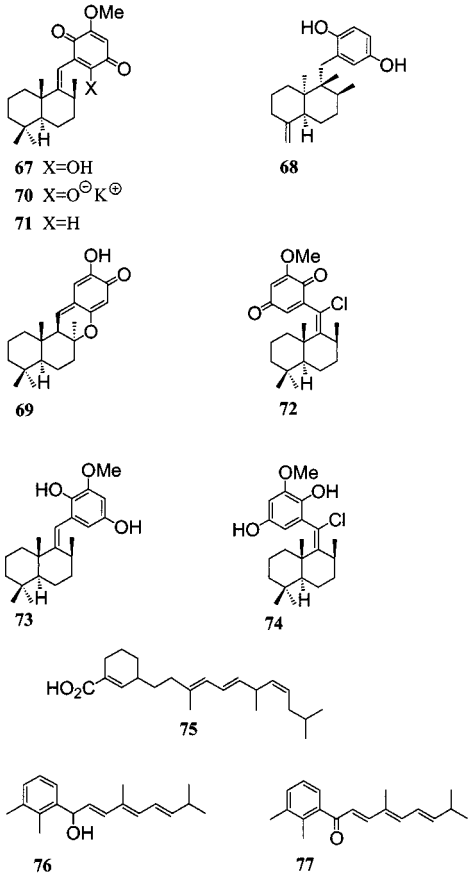
Biosynthetically related to the bisfurano cometins are the trisfurano hippospongins A−F (**60**−**65**) isolated as antibiotic agents from a *Hippospongia* sp collected during scientific trawling in the Great Australian Bight.[26] Sponges are also an established source of novel steroids. Illustrative of this capability is mycalone (**66**), a novel oxygenated sterone isolated from a specimen of a *Mycale* sp, collected by SCUBA off the mid-south coast of New South Wales.[27]

Mixed Terpene/Aromatic Biosynthesis

One of the more prolific classes of marine sponge metabolite are those of mixed sesquiterpene and "aromatic" biosynthesis, among the first examples of which were ilimaquinone (**47**)[24,28] and spongiaquinone (**67**).[29,30] With >120 examples of this structure class in the primary literature[31] these compounds can incorporate sesquiterpene units that



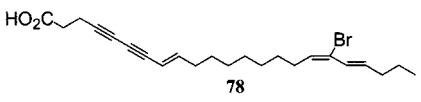
are acyclic, monocyclic, bicyclic or tricyclic, with aromatic subunits at either quinol or quinone oxidation levels. Typically detected and isolated due to their antibacterial activity, other biological properties that have been ascribed include inhibitory activity against HIV and more recently against the cholesteryl ester transfer protein. In addition to discovering new sesquiterpene/quinones from southern Australian sponges, we explored in some detail their acid-catalysed rearrangement and degradation.[32,33] These studies led to the reassignment [e.g. ilimaquinone (**47**)[24] and spongiaquinone (**67**)[30]] and assignment [e.g. arenarol (**68**)[33] and puupehenone (**69**)[34]] of absolute stereochemistry to many known examples of this structure class. Along the way we encountered an array of interesting structure variants, including spongiaquinone potassium salt (**70**) from a *Spongia* sp,[30] and the deoxyspongiaquinones **71** and **72**, and corresponding quinols **73** and **74** from a *Eurospongia* sp.[35] Both these sponges were collected during commercial and scientific trawling operations in the Great Australian Bight, respectively. Structure assignments were supported by detailed spectroscopic analysis, including NOE difference measurements, as well as chemical interconversion and degradation. Most recently we discovered the first example of this structure class where the aromatic subunit retained a nonaromatic character consistent with the possible involvement of shikimate in the biosynthesis.[36] While the involvement of shikimate in the biosynthesis of sesquiterpene/quinones has been speculated, the discovery of clathrin A (**75**) offers additional support for this hypothesis. Clathrin A (**75**) was isolated along with the novel co-metabolites clathrin B (**76**) and C (**77**) from a *Clathria* sp collected during scientific trawling operations in the Great Australian Bight.[36] Clathrin B (**76**) proved unstable and during handling underwent oxidative conversion into clathrin C (**77**), suggesting that this latter compound may be an artefact of the isolation procedure.



Lipids

Carduusynes A−E are antibacterial acetylenic lipids isolated from a specimen of *Phakellia carduus* obtained during commercial trawling operations in the Great Australian Bight.[37] Carduusyne B (**78**) incorporates unusual conjugated diyne-ene and bromo diene moieties. Carduusyne E (**79**), on the other hand, features conjugated diyne-ene and diyne moieties, the latter augmented by a propargylic alcohol. Empirical analysis of the ^{1}H NMR chemical shifts for both diastereomeric *O*-methylmandelate esters of **79** was used to assign absolute stereochemistry about the chiral propargylic alcohol. Careful analysis of these data revealed carduusyne E (**79**) to exist as an 87:17 ratio of *S* and *R* enantiomers. This enantiomeric mixture was determined to be natural since carduusyne E (**79**) did not undergo further racemization on repeated exposure to the isolation conditions, or during chemical derivatization. This observation highlights the potential for less than 100% optical purity in

the case of chiral natural products featuring only a single chiral centre, whether this be natural or an artefact of handling, and the need to employ analytical procedures capable of diagnosing these events when they occur. Even greater caution would be required when the single chiral centre is labile and capable of partial or total racemization (consider the case of the echinosulfonic acids described below).



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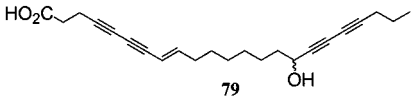
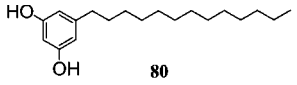


79

A selection of eleven alkyl and alkenyl resorcinols was isolated from a *Haliclona* sp collected by SCUBA off Flinders, along the Bass Strait coast of Victoria.[38] These included grevillol (**80**), a known natural product from Australian terrestrial bushes of the genus *Grevillea*,[39] and from the Australian marine brown algae *Cystophora torulosa*[40] and *Caulocystis cephalornithos*,[41] as well as the new saturated and unsaturated analogues such as **81** and **82**. Alkyl and alkenyl resorcinols are known for their vesicant or blistering properties, and presumably offer a measure of chemical defence (antifeedant and/or antifouling) for the producing organism. Consistent with this hypothesis, the sample of *Cystophora torulosa* observed to be rich in resorcinols was noted to be "surprisingly....devoid of hydroid symbionts".[40] The blistering properties of alkyl resorcinols have also been employed as scarifying agents in the tribal wound rituals of some Australian aborigines, and were responsible for the discomfort of some early Australian explorers who incautiously experimented with *Grevillea* sp as a substitute for tea and coffee.[38]



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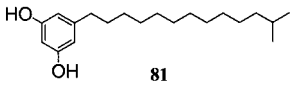

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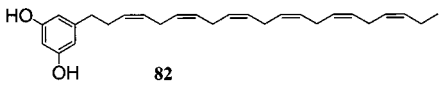
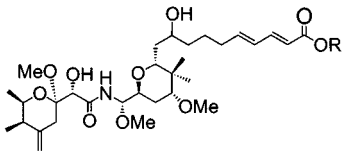
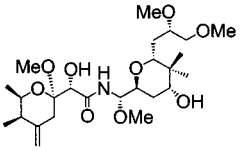


82

The heavily functionalized lipid onnamide F (**83**) was isolated as the nematocidal agent in a *Trachycladus* sp collected by SCUBA in Port Phillip Bay, Victoria.[42] As with other marine metabolites mentioned in this review, onnamide F (**83**) was acid sensitive and underwent ready decomposition on handling or storage in aprotic solvents. Storage in protic solvents, or conversion into the methyl ester **84**, stabilized the molecule. Both the natural carboxylic acid **83** and it ester derivative **84** displayed potent nematocidal properties. It is interesting to note that onnamide F (**83**) displays structural similarities with pederin (**85**), which is the chemical defence agent of the blister beetle. It is plausible that onnamide F (**83**) also has ecological value as a chemical defence agent.



83 R=H
84 R=Me

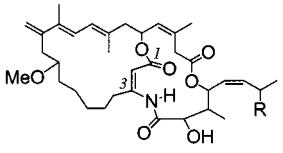


85

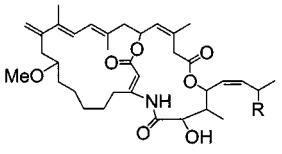
Macrolides

In recent years our exploration of the chemistry of southern Australian marine sponges has been directed towards the discovery of compounds with commercial potential as agrochemicals. Very specialized high throughput bioassays identify target extracts and support the bioassay-directed isolation of metabolites with activity against endo (nematodes) and ecto (flies, fleas and ticks) parasites. Early discoveries from this program include the nematocidal onnamide F (**84**) (see above),[42] as well as the amphilactams A−D (**86**−**89**), and geodin A Mg salt (**90**). The amphilactams are novel macrocyclic acyl enamino lactams that were isolated from two specimens of the genus *Amphimedon* collected during separate scientific trawling operations in the Great Australian Bight.[43] To our surprise the acyl enamino lactam/lactone functionality at the core of all amphilactams proved to be unprecedented in the natural products literature, and was the subject of only cursory comment in the chemical literature as a whole. The spectroscopic characteristics of this functionality were sufficiently unusual [i.e. for amphilactam A (**86**) ^{13}C NMR: C-2: $\delta = 89.5$ and C-3: $\delta = 158.6$] to warrant synthesis of the model compound **91**. This model was prepared in quantitative yield in one step from the *p*TsOH catalyzed condensation of methyl 3-oxobutanoate with acetamide, and provided an excellent spectroscopic match with amphilactams A (**86**) and B (**87**). The common *Z* $\Delta^{2,3}$ stereochemistry for **86**, **87** and **90** was readily determined from the deshielded ^{1}H NMR resonance for the H-bonded lactam/amide NH ($< \delta = 10$). By contrast this same proton resonated ≈ 4 ppm upfield in amphilactams C (**88**) and D (**89**), where an *E* $\Delta^{2,3}$ stereochemistry precluded H-bonding. The geodin A Mg salt (**90**) was isolated from several specimens of *Geodia* sp collected during scientific trawling operations in the Great Australian Bight.[44] While a number of antibiotic macrocyclic lactams related to **90**

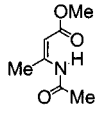
have been reported from marine and terrestrial microbes, it is noteworthy that none have been isolated as Mg salts. A careful examination of the literature revealed that all prior isolations involved acidification either during extraction or chromatography. Consequently, it is impossible to know if any of these known compounds exist naturally as Mg salts. This oversight becomes more significant given our observation that the free acid of geodin A Mg salt is unstable. Repeated attempts to convert geodin A Mg salt (**90**) into its more soluble acid form, for the purpose of spectroscopic comparison with model compounds, revealed the cyclopentenyl diene portion of the molecule to be highly susceptible to oxidation — leading to a complex mixture of products. Had we inadvertently converted geodin A Mg salt into the free acid during isolation and handling, it is doubtful whether the structure elucidation would have proved successful. Whether other acid-labile macrocyclic lactams have eluded detection due to decomposition remains unknown, but our experiences with geodin A Mg salt would counsel against acidification during isolation, at least without careful analysis of the consequences.



86 R = H
87 R = Me



88 R = H
89 R = Me



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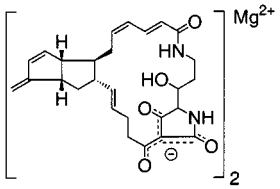
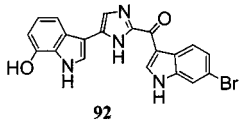


90

Alkaloids

A common structural motif among marine alkaloids is that of the bromoindole. The bromoindoles isobromotopsentin (**92**) and dragmacidin E (**93**) were isolated, along with the dimethylhypoxanthine **94**, from a *Spongosorites* sp collected during scientific trawling operations off the coast of South Australia.[45−47] Isobromotopsentin (**92**) is a member of an extensive family of related dipeptides, various examples of which have been reported to possess cytotoxic, antitumour, antiviral, antifungal and/or antiinflammatory activity. By contrast, the only close natural analogue to the tripeptide dragmacidin E (**93**) is its co-metabolite dragmacidin D (**95**). Dragmacidin E (**93**) was detected and isolated due to its ability to inhibit protein phosphatase enzymes, and presented an interesting challenge in structure elucidation. Whereas many marine metabolites are acid sensitive, without addition of acid (TFA) the NMR spectroscopic data for **93** were excessively broadened and unable to support unambiguous structure assignment. This broadening completely suppressed ^{13}C NMR resonances for C-3 and C-6. On addition of TFA the pyrazine/pyrazinone tautomeric equilibrium was modulated to the point where all ^{13}C NMR resonances could be observed. The need to observe these ^{13}C NMR resonances was all the more significant given the considerable number of contiguous quaternary centres, and the heavy reliance on gHMBC analysis. The dimethylhypoxanthin **94** was also rich in quaternary centres, and unambiguous structure assignment was eventually only possible by interpretation of ^{1}H-^{15}N gHMBC data.



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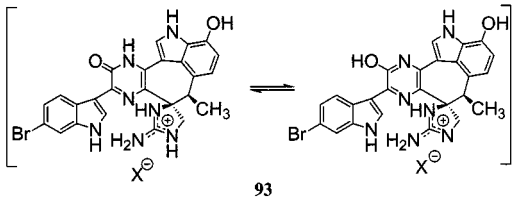

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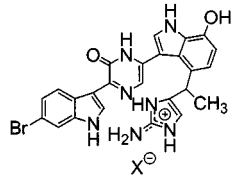

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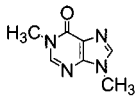


94

The psammopemmins A−C (**96−98**) are novel metabolites isolated from a *Psammopemma* sp collected during scientific trawling operations in Prydz Bay, Antarctica.[48] In addition to being rare 4-hydroxyindoles, the psammopemmins incorporate an unprecedented 2-bromopyrimidine moiety. The echinosulfonic acids A−C (**99−101**) and echinosulfone A (**102**) are novel antibacterial bromoindole dimers isolated from an *Echinodictyum* sp collected during scientific trawling operations in the Great Australian Bight.[49] During extraction, isolation and handling the echinosulfonic acids were prone to interconversion through solvolysis. This reactivity is dramatically illustrated by addition of acid, during which the echinosulfonic acids undergo a reversible colour change from pale yellow to purple, presumably due to formation of the resonance-stabilized carbocation shown in Figure 4. Quenching of this intermediate carbocation with water, MeOH or EtOH can lead to the racemic echinosulfonic acids A−C (**99−101**). The sulfonic acid and sulfone functionality observed in these *Echinodic-*

tyum metabolites are extremely rare, and their biosynthetic origins remain a mystery.

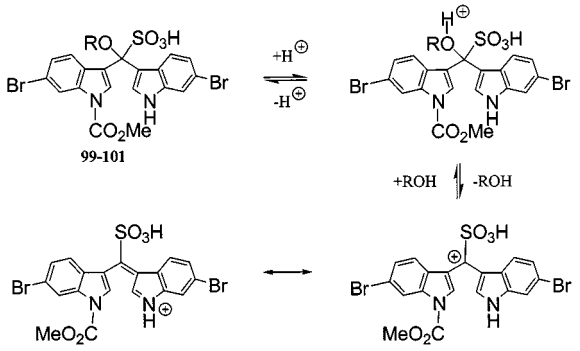
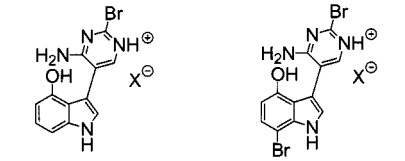
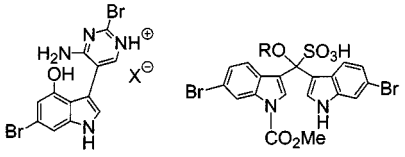
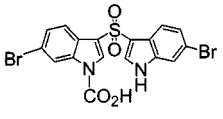


Figure 4. Purple species (carbocations) produced on addition of acid to echinosulfonic acids A−C (**99−101**)





99 R=Et
100 R=Me
101 R=H

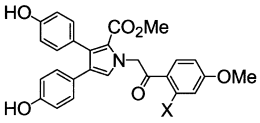


Continuing the theme of bromo alkaloids are a series of bromo pyrroles isolated from an *Axinella* sp collected during scientific trawling operations in the Great Australian Bight.[50] Included among the metabolites isolated from this sponge were the dibromopyrrole carboxylic acids **103−106**, and the tribromopyrrole carboxylic acid **107**. The latter compound was only present in trace amounts and, not surprisingly, was difficult to detect by NMR techniques. Unambiguous assignment of these structures, including bromo regiochemistry, was secured by total synthesis.

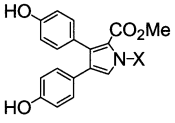
103 R=H
104 R=Me
105 R=Et
106 R=$CH_2CH{=}CH_2$

107

Other pyrrole alkaloids from southern Australian marine sponges are the lamellarins. Lamellarins O−P (**108−109**) were isolated from a specimen of *Dendrilla cactos* obtained during scientific trawling operations in Bass Strait,[51] whereas lamellarins Q−R (**110−111**) were isolated from another specimen of *Dendrilla cactos* collected by SCUBA off the mid-south coast of New South Wales.[52] The lamellarins as a class of marine metabolite were first observed from a marine mollusc, and then from marine didemnid ascideans. Their discovery in sponges suggests a biosynthetic origin that may involve microbial symbionts. In addition to antibacterial properties, some lamellarins have been attributed with activity against HIV-1, and also against multiple drug resistant (MDR) tumours. These activities have prompted interest in the synthetic supply of lamellarins and analogues. Isolation of a microbial source for the lamellarins could provide a renewable resource from which to explore their biological and commercial potential.



108 X=H
109 X=OH



110 X=H
111 X=p-phenol

In addition to novel metabolites featuring exotic functional groups, some marine natural products are surprising for other reasons. Discovery of the commercial solvent sulfolane (**112**) in a sponge/tunicate composite (*Batzella* sp/*Lissoclinum* sp)[53] was so unexpected that a recollection was undertaken to confirm this finding. Likewise, the discovery of extremely high levels of Cd (15,000 ppm) and Zn (5,000 ppm) salts as the bioactive principles in collections of the Antarctic sponge *Tedinia charcoti* warranted confirmation.[54] Several specimens of *Tedinia* obtained during scientific trawling operations off the Antarctic coast have since been confirmed to be rich in Cd and Zn salts. The mechanism for bio-accumulation of these inorganic *chemical defence* agents remains a mystery.

112

Conclusion

This Microreview has attempted to illustrate the molecular virtuosity of marine organisms, albeit through a single phyla — sponges. The molecular diversity that we have encountered in our investigations into southern Australian (and Antarctic) marine sponges (as well as algae and tunicates) is extraordinary, and the examples presented above are but a selection of the novel structures that have emerged during these studies. Many projects in progress in our laboratories are targeting new and ever more exotic marine metabolites, some with exciting biological properties. Many

other specimens within our collection have tested positive in screening programs aimed at detecting commercially significant biological activities, and a significant number of extracts still await detailed chemical investigation. Add to this the demonstrated potential of marine organisms other than sponges (molluscs, ascideans, soft corals, bryozoa, algae, microbes...), as well as the large number of marine ecosystems yet to be sampled, and the increasing power of new technologies in isolation and spectroscopic analysis, and the future of marine bioprospecting is exciting indeed.

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