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Marine Natural Products Chemistry: Past, Present, and Future

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In accepting an invitation to write this foreword this seemed to be an excellent opportunity to acknowledge some of the seminal achievements in Marine Natural Products Chemistry (MNP) over the past 60 years. With a wealth of material to choose from the task promised to be rewarding and straightforward. Unfortunately the promise proved illusory, as it quickly became clear that a foreword was not the vehicle to do justice to the many and varied achievements that appear under the banner of MNP. Fortunately, such compilations of achievement can be found in several excellent reviews, books, and databases. Instead, and in the interests of providing a different perspective on MNP, this foreword steps back from the specifics to consider the field more globally, reflecting on the trends that have guided, challenged, and shaped MNP from the pioneering achievements of last century, to where we stand today.

To the uninitiated the term *Marine Natural Products Chemistry* seems to describe a narrow sub-disciplinary branch of natural products chemistry, however, modern MNP operates well beyond this literal interpretation. Over the past half-century MNP has evolved into a multidisciplinary endeavour, rich in complementary technologies, methodologies, and objectives, with the schematic in Fig. 1 illustrating some aspects of this connectivity. In its broadest sense MNP can be viewed from the twin perspectives of basic and applied science. The basic science of MNP embraces discrete and yet interconnected investigations into what metabolites are made, how they are made, by which marine taxa, from where, under what conditions and to what ecological purpose – with an emphasis on discovering and understanding molecular novelty and diversity. MNP basic science is a curiosity-driven knowledge-trawling endeavour, fully and freely disclosed for the common good. By contrast, the applied science of MNP undertakes the core chemical investigations as described above for basic science, but prioritized through the filter of commercially relevant biological activity. Often referred to as *Marine Biodiscovery*, MNP applied science represents a more tightly focussed search for marine metabolites and associated pharmacophores that have potential value in human or animal health, crop protection, or as research tools (bioprobes). The transition from curiosity (basic) to commercial (applied) imposes significant constraints on public disclosure, as discoveries are first assessed in confidence for possible protection and development as intellectual property (IP). Notwithstanding the basic versus applied dichotomy, MNP enjoys a healthy level of crossover benefit, with some researchers and laboratories operating seamlessly and successfully to achieve both basic and applied outcomes. The remainder of this foreword represents a personal analysis of the trends that have shaped and defined MNP, across both basic and applied science, keyed against the schematic outlined in Fig. 1.

Marine Natural Products Chemistry

MNP had its origins in and remains heavily influenced by organic chemistry. Pioneering MNP chemists enjoyed the excitement and challenge of detecting, isolating, characterizing, identifying, evaluating, and otherwise exploring marine metabolites ranging across all biosynthetic classes, including some unique to the marine environment. These metabolites featured remarkable structural diversity inclusive of rare carbon and heterocyclic skeletons, and complex functionality. Unlike terrestrial counterparts, marine metabolites were quickly recognized as being less stable and in need of added care during handling and characterization, avoiding excessive exposure to light, heat, oxygen, and variations in pH. Historic methodologies used to study plant alkaloids (acid and base extraction) or essential oils (steam distillation) did not translate well to MNP. The introduction of high performance liquid chromatography (HPLC), supported by a wealth of reverse-phase media, and refractive index (RI) and variable wavelength ultraviolet-visible light (UV) detectors, was a key innovation. Subsequent advances from isocratic to gradient HPLC, employing integrated in-line diode array (DAD), evaporative light scattering (ELSD) and mass (MS) detectors, was pivotal in accelerating MNP, leading to the discovery of legions of metabolites, from ever-smaller biodiversity samples. The technological timing was opportune, as the logistical challenges associated with marine collecting, including restricted

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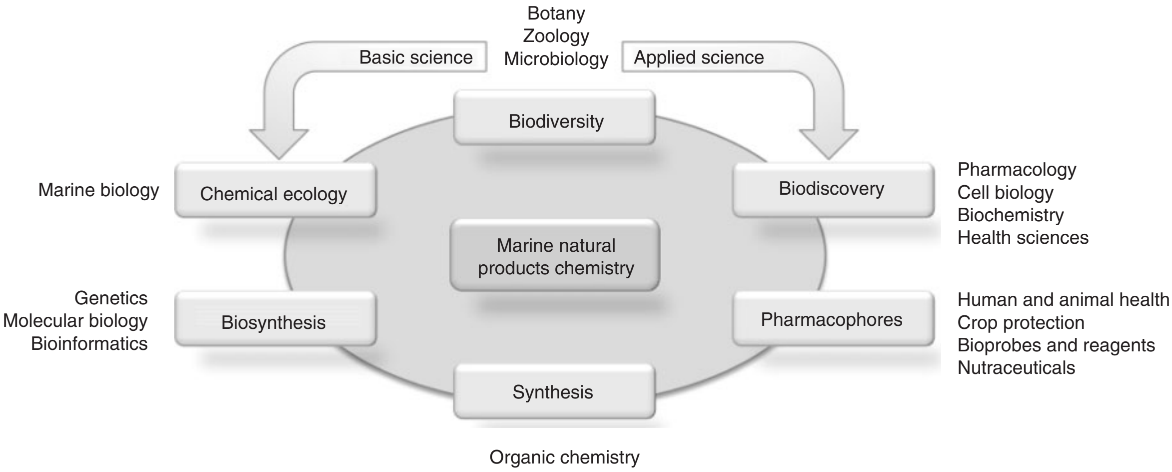

Fig. 1. Schematic of the broad field of marine natural products.

underwater bottom-time, and the rarity, in situ cryptic nature and small physical size of some taxa, hampered and even precluded recollection. Modern MNP researchers acquired the skills to complete individual chemical investigations with recourse to only a single small specimen – radically increasing the accessible biodiversity pool, reducing costs and minimizing environmental impact. Over the decades the minimum mass of natural product required for structure elucidation plummeted, such that many modern MNP laboratories routinely solve structures on sub-mg quantities. Critical to this 'sensitivity' revolution was the shift from combustion analysis to high-resolution mass spectrometry for assignment of molecular formula, and an extensive reliance on high field NMR with increasingly specialized probe assemblies and pulse sequences. Often overshadowed by laboratory based instrumental wizardry, reliable and routine desktop access to databases such as SciFinder and Web of Science, and entire online journal and library collections, supportive of instantaneous online text, molecular structure (and sub-structure) and chemical property searching, fuelled an information revolution across all fields of science. Today we (MNP researchers and others) can achieve more, faster, and with far less.

Biodiversity

Access to marine biodiversity is perhaps the most obvious defining feature and constraint on MNP, and explains why this field historically lagged well behind terrestrial plant natural products chemistry – with marine biota safely submerged out of sight and out of mind. Ready access mid last century to self-contained underwater breathing apparatus (SCUBA) provided a key enabling technology that opened a window to MNP, allowing researchers for the first time to roam the underwater world and observe at first hand benthic marine biodiversity in situ. For those with bigger budgets, dedicated research vessels, submersibles, and a willingness and ability to travel by air/sea to and from remote locations across tropical, temperate, and polar climes brought a bewildering array of marine biodiversity into laboratories. Not surprisingly, many early biodiversity choices were influenced by ease of collection, with investigations into intertidal red (Rhodophyta), brown (Phaeophyta), and green (Chlorophyta) algae featuring prominently. Likewise, sponges, soft corals, gorgonians, and tunicates caught the attention of MNP researchers. As relatively large sessile benthic species to be found within the intertidal/SCUBA zone (0–30 m), these organisms were an ideal size for chemical analysis, and were so obvious in the field that even a chemist could bag and tag. As noted above, improvements in instrumentation and sensitivity made smaller taxa scientifically attractive – with molluscs (*Aplysia*, *Nudibranchia*, *Siphonaria*, cone shells, etc.) and various other small or encrusting organisms (including egg masses) being added to the sample bag. It is worthwhile noting that one of the many collateral scientific returns accrued through MNP has been support for, and growing interest in, marine taxonomy and systematics. Notwithstanding these successes, the major growth in MNP over the past decade has been in marine-derived microbial biodiversity – bacteria and fungi – the ultimate in small biodiversity. This latter trend is somewhat ironic given that arguably the first modern scientific account of a marine natural product was a 1950s report on the antibiotic cephalosporin C, from the fungus *Cephalosporium acremonium* obtained from a sewer outfall off the coast of Sardinia. Despite that early marine-derived fungal success, it took almost a half-century for microbes to gain traction in MNP. Possible reasons behind the (re) emergence of marine microbes as a valued biodiversity source are worth consideration. With increased exploration of macro marine organisms the incidence of rediscovery of known chemistry increased, encouraging and even requiring that researchers look further afield. At the same time, speculation that related chemistry encountered in taxonomically distinct macro marine organisms was due to symbiotic or commensurate microbes drew attention to the biosynthetic potential of marine microbes. Reinforcing the importance of this potential, it became increasingly apparent that many metabolites isolated from macro marine organisms could not be supplied in sufficient quantity – not without unacceptable cost and environmental impact – delaying or stalling biological investigations. The belief that fermentation and cultivation of marine microbes offered a practical solution to all three of the challenges listed above became widely accepted. If these drivers were not enough, published accounts of technical success in isolating and cultivating marine-derived (and obligate) microbes, coupled to a growing catalogue of novel marine microbial metabolites, only strengthened the case. Parallel to these scientific drivers, other factors influenced the move by some, from macro to micro. The regulatory environment in which modern MNP operates is a far cry from the relaxed regimes of yesteryear. National and regional jurisdictions increasingly regulate access through collection and export permits, with some demanding formal benefit sharing agreements (well ahead of a benefit to share). Even where approval is given, MNP researchers must be ever mindful and respectful of indigenous knowledge and cultural sensitivities. If this added administrative and sociological burden was not enough, heightened awareness of occupational health and safety issues, coupled with risk averse university administrations, imposed additional hurdles that in some cases precluded the informal scientific diving that served generations of research higher degree students and MNP so well. Faced with an attractive alternative scientific opportunity, and the not unreasonably desire to minimize regulatory load, many MNP researchers have seen merit in and made the transition to collect sand and sediment, to isolate, culture and study the microbes within. In reality (as discussed below) both macro and micro marine biodiversity continue to present enormous untapped molecular potential, more than capable of inspiring significant future discoveries.

Chemical Ecology

Marine chemical ecology seeks to better understand the interactions between marine metabolites, the producing/host organism, surrounding communities and marine ecosystems. Conventional wisdom has marine natural products providing host organisms with a survival advantage, typically referred to as a chemical defence. The host organisms can themselves be the biosynthetic source of these metabolites, or can acquire them from dietary sources and/or symbiotic/associated organisms (bacteria or microalgae). These chemicals can protect the host from infection (antibiotics, antiparasitics), repel or dissuade predators (antifeedants, toxins), inhibit the development and growth of competitors (selective cytotoxins and cell growth inhibitors), or even guard against UV radiation (sunscreens). They may also enhance reproductive outcomes (sperm attractants), or improve the ability to feed by rapidly immobilizing prey (venoms). This ecological significance can on occasion be tested experimentally. For example, a marine algal metabolite that elicits a feeding avoidance response when added to the food pellets of aquaria fish might have an ecological role as an antifeedant against herbivorous reef fish. Similarly a marine tunicate metabolite that is antibacterial towards laboratory strains of bacteria might protect the tunicate from opportunistic pathogens present in seawater, while a marine sponge metabolite that selectively kills fast growing cancer cells in tissue culture might inhibit growth and development of the avalanche of larval species that threaten overgrowth of filter feeding organisms such as sponges. The logistical challenges associated with testing these hypotheses and studying marine chemical ecology in the field are imposing, and are largely the domain of a select sub-group of specialist MNP researchers. As knowledge of marine metabolites has grown to include many thousands of diverse structures from numerous species, consistent with the chemical defence hypothesis, it has become clear that these chemicals are disproportionally represented in sedentary or slow moving lineages such as invertebrates and algae. As these life forms typically lack the physical attributes to flee (fins, legs), fight (claws, spines), or take cover (shells), and include some of the oldest marine life forms, it is little wonder that they have experienced both the selection pressure and time to evolve chemicals to enhance survival. Similarly with marine microbes, which much like their terrestrial cousins have embraced chemistry to enhance survival. Whereas the early days of marine chemical ecology were dominated by investigations designed to reverse engineer the ecological role of selected *known* metabolites, current trends are extending towards *de novo* chemical evolution, with MNP researchers mapping the incidence and function of secondary metabolism genes, and unravelling the environmental cues and pathways associated with their transcription to produce both *known* and *unknown* marine metabolites, and elicit ecological responses. The future holds out the promise that chemical ecology may lead, rather than follow, the discovery of new metabolites.

Biosynthesis

The early years of MNP biosynthetic studies were dominated more by speculation of biosynthetic origins, than experimental evidence. Pioneering MNP researchers recognized the familiar carbon frameworks and biosynthetic signature of terpenes, polyketides, peptides, and more, as well as a wealth of metabolites of mixed, and some of unknown biosynthetic origin. One of the earliest uniquely marine biosynthetic challenges was rationalizing the over abundance of brominated rather than chlorinated marine metabolites. Drawing on fundamental chemical theory and experimental evidence, the solution of haloperoxidases and halonium chemistry quickly acquired textbook status. Notwithstanding this success, the application of traditional radiolabel feeding studies to marine biota, to track and quantify incorporation of key biosynthetic precursors and unravel biosynthetic pathways, imposes enormous technical challenges. It is testament to the perseverance and ability of specialized MNP researchers that several such studies have been successful. In more recent years the focus on MNP biosynthesis has grown to include molecular biology and genetics. Not content to speculate on biosynthetic pathways of known metabolites, MNP researchers are sequencing, annotating and even reshuffling and manipulating selected pathways, particularly polyketide synthases. While such studies have by necessity been restricted to organisms whose genome has been sequenced (i.e. selected marine bacteria), the extraordinary advances that continue to be made in genome sequencing and annotation suggest that in the very near future this approach will be extended to an ever wider selection of marine biota.

Synthesis

Natural products and synthetic chemistry have traditionally been strong allies. In this relationship, natural products chemists discover and present evidence for novel natural products, often possessing an exotic array of carbon skeletons, heterocycles, and functionality. For their part synthetic chemists use these novel structures as platforms to develop new synthetic methodology, and in the process confirm (or revise) assigned structures. This alliance is all the more significant where the target natural product is in limited supply, has exciting and potentially very valuable properties, and/or where the structure assignment is incomplete or in doubt. With improved knowledge and appreciation of the biosynthesis of marine metabolites, synthetic chemists are increasing recognizing and employing biomimetic strategies. The wealth of structurally diverse marine metabolites continues to provide synthetic chemists with an endless supply of inspiring structures.

Biodiscovery

Marine metabolites represent an extraordinary pre-assembled pool of biologically active molecular diversity, programmed by evolutionary processes to be potent and selective modulators of key biopolymers (i.e. DNA, proteins, etc.), cells, tissues, organs, and animals. In essence these metabolites represent Nature's intellectual property, gleaned from the evolutionary equivalent of a billion year global drug discovery program, boasting an unlimited budget and a workforce of trillions. Clearly a very, very, long-term investment, but one with an impressively successful intellectual property (IP) portfolio! The privileged biologically active chemical structures that have emerged from this (ongoing) investment can inform, guide, and inspire modern drug discovery, allowing us to re-purpose ecological advantage to pharmaceutical benefit. The strategies that MNP researchers have employed to discover valuable biologically active metabolites has evolved and matured over time. Early biodiscovery efforts were dominated by simple bioassays that aimed to detect antibiotics (i.e. disc diffusion) or anticancer agents (i.e. cytotoxicity). With the benefit of hindsight, many of these early screening strategies were somewhat naïve, lacking in clinical relevance, or the mode-of-action or in vivo studies critical to delivering promising drug lead candidates into the drug discovery pipeline – and might be better characterized as enhanced basic science, rather than applied science. As harsh as this assessment may seem, enhanced MNP basic science nevertheless played a pivotal role, facilitating the discovery of a great many interesting natural products, and most importantly laying the foundation for modern MNP biodiscovery. Building on these formative experiences, modern MNP researchers have acquired expertise and experience in both the science and the business of biodiscovery – building interdisciplinary collaborative research networks, engaging with industry, applying clinically relevant bioassays, targeting unmet medical need, and acquiring valuable practical knowledge of the drug discovery pipeline. By necessity MNP researchers have become conversant with *material transfer agreements*, *confidentiality agreements*, and *collaborative research agreements*, and the concepts of IP, royalties, milestones, patents and, of course, venture capital. The blooding of MNP biodiscovery researchers in the terminology and corporate culture of biobusiness was a critical growth experience that has paid dividends in the quality and calibre of modern efforts and achievements.

Pharmacophores

In recent years advances in biology have identified and defined important signalling, transport and developmental pathways, leading to a bewildering array of novel molecular targets, keyed to specific diseases. Similarly, advances in structural biology have visualized the intimate inner workings of the substrates and receptors that make up many of these targets, highlighting functional homology beyond peptide sequences and across species, while high sensitivity, high throughput and high content bioassay technologies have rendered these targets to 'discovery' practice. New assay technologies and methodologies have empowered MNP researchers to redefine the anticancer and antibiotic discovery paradigms of the past, while simultaneously reaching out to address neurodegenerative diseases, obesity, inflammation, pain, diabetes, cardiovascular disease, anxiety, and more. This expanded palette of bioassays has driven the discovery of new chemistry, as well as the rebirth of known marine metabolites, finding application and purpose where previously there was none. With improved sensitivity and access to even the most minor co-metabolite, MNP researchers can immediately and rapidly accelerate structure–activity relationship (SAR by co-metabolite) investigations, refining and defining pharmacophores, building and strengthening IP positions, and advancing marine metabolites into the drug discovery pipeline. No longer mere indivisible and disconnected molecular stamps, marine metabolites are increasingly recognized as exemplars of valuable molecular motifs.

In Conclusion

As noted above, the early history of MNP was dominated by a collegiate community of academics, pursuing largely curiosity driven research, targeting metabolites with novel chemical structures. This golden age of enquiry (basic) science was pleasantly uncomplicated. In more recent years, MNP has evolved such that valuable biological activity and drug discovery have emerged as the prism through which 'success' is measured – both in terms of research funding (grants, contracts) and outcomes (papers, patents). In keeping with this shift, collaborations across disciplinary boundaries (chemistry, microbiology, ecology, pharmacology, cell biology, molecular biology, bioinfomatics, etc.), awareness of and attention to market and community need (therapeutics, nutraceuticals, agrochemicals, etc.), and engagement with industry, have become prized attributes of successful MNP programs and researchers. Modern MNP enjoys a broadened profile with significant successes, but not without challenges. In addition to the technical, intellectual and funding imperatives that define modern science, today's MNP researchers must contend with the dichotomy of publish (share knowledge) versus patent (protect knowledge), restrictions on the collection and transport of biodiversity, and legislative requirements at local, national, and international levels. That the latter is driven by an enthusiasm to control and ultimately derive a national (financial) benefit from biodiversity reserves is perhaps understandable. Notwithstanding the logic of this case, MNP researchers have a responsibility to educate on the distinction between a '*potential for*' versus an '*expectation of*' commercial returns (a subtlety all too easily overlooked by those who lack the laboratory and research perspective, but nevertheless control access and funding, and influence community opinion). In 60 years MNP has come a long way, and the prospects for future discovery, while different, can be as bright as ever. There is every reason to be cautiously optimistic.

To conclude with a nautical metaphor,

> '. . . Marine metabolites can be viewed as Nature's molecular waypoints, guiding our exploration of the infinite reaches of marine chemical space, serving as both inspiration and compass as we search out and explore the elusive archipelagos of biosynthetic diversity, ecological purpose and biological activity, and seek to map the rare and hidden safe harbour's of new pharmacophores and drugs. The MNP ship may have set sail, but the voyage is far from over.'